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A review

BY

JENS LYNCSØF

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SERUMINSULIN

FROM THE SECOND UNIVERSITY CLINIC OF INTERNAL MEDICINE
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A review

BY
JENS LYGSOE

ÅRHUS 1965

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Authors Previous Publications on This Subject

- 1 Determination of the Insulin like Activity in Serum Using Rat Epididymal Adipose Tissue *Scand J Clin Lab Invest* 13 628-636 1961
- 2 The Insulin like Activity in Serum Determined by the Rat Epididymal Fat Method I Normal Values in Undiluted and Diluted Serum and the Effect of Ingestion of Glucose *Acta Med Scand* 171 365-375 1962
- 3 The Insulin like Activity in Serum Determined by the Rat Epididymal Fat Method II The Values in Undiluted and Diluted Serum from Diabetic Patients Determined before and after the Ingestion of Glucose *Acta Med Scand* 172 41-62 1962
- 4 The Insulin like Activity in Serum Determined by the Rat Epididymal Fat Method III Insulin Antagonism in Serum from Normal Persons and Diabetics Including some Observations on Pigs Blood *Acta Med Scand* 172 601-614 1962
- 5 The Insulin like Activity in Serum Determined by the Rat Epididymal Fat Method IV Anti insulin Inhibition of Insulin like Activity in Electrophoretically Separated Serum Protein Fractions *Acta Med Scand* 174 582-594 1963
- 6 The Insulin like Activity in Serum Determined by the Rat Epididymal Fat Method V The Distribution of Insulin like Activity in Electrophoretically-separated Serum Protein Fractions from Normal Fasting Subjects and the Effect of Ingestion of Glucose *Acta Med Scand* 175 401-408, 1964



PREFACE

The present work was carried out during my employment at the Second University Clinic of Internal Medicine, Kommunehospitalet, Århus, Denmark. Head Professor Knud Lundbæk, M.D.

The thesis is based on investigations made during the years 1959 to 1963.

To Professor Knud Lundbæk I wish to express my profound gratitude for ideal working conditions and encouragement. Professor Lundbæk's great interest in the subject of this study has contributed much to the completion of this work.

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Copenhagen, October 1965

Jens Jørgen

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INTRODUCTION

The elucidation of the pathogenic factors in diabetes mellitus and other diseases which probably involve abnormalities of insulin production, has been hampered by methodological problems in the determination of the insulin content of the serum. The methods which have been elaborated for this determination are technically very difficult, and different investigators have obtained highly divergent results, even when one and the same method was used. For years, no satisfactory explanation could be found for the lack of agreement between serum insulin values obtained in these investigations. It is only in the last few years, with the publication of studies suggesting that serum contains several

different forms of insulin, that there has been hope of a satisfactory theory emerging to explain the divergences mentioned. At the same time, an understanding of the mode of secretion of insulin also appears to be within reach, such an explanation is a prerequisite for elucidating the pathogenesis in diabetes mellitus and in the different hypoglycaemic states.

In the present study, the literature on serum insulin has been covered till the autumn of 1963. Previous summaries of the literature have been made by Willebrands & Groen (1954) (293), Vallance Owen & Wright (1960) (279), and Yalow & Berson (1960) (309).

Definitions and abbreviations

- Insulin like activity (ILA)* the activity which can be measured by a biological method for determining insulin
- Serum insulin like activity (SILA)* the activity which can be measured by a biological method in untreated, undiluted or diluted blood, serum and plasma
- Immunologically determined insulin* the activity which can be measured by an immunological method for determining insulin
- Hidden ILA* the insulin like activity which, in untreated undiluted or diluted serum, is biologically and/or immunologically inactive
- Suppressible SILA* that part of SILA (measured by the rat epididymal fat method) which is inhibited after addition of anti insulin
- Non suppressible SILA* that part of SILA (measured by the rat epididymal fat method) which is not inhibited after addition of anti insulin
- Insulin antagonist* serum factors which in vitro inhibit the effect of insulin on an isolated tissue
- A fraction* ILA localized to albumin- α_1 globulin
- B fraction* ILA localized to β γ globulin

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Insulin like activity (ILA) the activity which can be measured by a biological method for determining insulin

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Insulin antagonist serum factors which in vitro inhibit the effect of insulin on an isolated tissue

A fraction ILA localized to albumin α_1 globulin

B fraction ILA localized to β γ globulin

TABLE I
In vivo techniques for the determination of small quantities of insulin

Parameter	Administration	Parameter tested	Sensitivity (μU/ml)	Precision (mean Lambda)	Reference
Intact mice	SC	Convulsions	2000-4000	-	Stewart (1960) ¹⁴
Intact mice	IV	Fall in blood sugar	1000	0.52	Begelman (1958) ¹²
Intact rats	IV	do	600	0.15	Arguilla et al (1962) ¹¹
Intact rats	II	Glycogen synthesis in the diaphragm	100	0.33	Rafaelson (1961) ¹³
Adrenalectomized mice	SC	Convulsions	2000	-	Hemmingsen & Olsen (1938) ¹¹⁹
Hypophysectomized rats	IV	Fall in blood sugar	1000	0.22	Anderson & Wilery (1962) ¹
Adreno-demethylated hypophysectomized rats	IV	do	300	-	Gellhorn et al (1941) ¹¹
do	IV	do	200	0.16	Hosaka et al (1963) ¹¹⁴
Alloxan-diabetic hypophysectomized rats	IV	do	2000	-	Randle (1954) ¹¹⁸
do	IV	do	2,000	0.31	Begelman et al (1956) ¹²
Alloxan-diabetic hypophysectomized mice	IV	do	1000-2000	-	Coetz et al (1954) ¹¹⁹
do	IV	do	2,000	0.70	Anderson et al (1957) ¹
Alloxan-diabetic adrenalectomized mice	IV	do	1000	-	Dard & Bornstein (1959) ¹²
Adreno-demethylated alloxan-diabetic hypophysectomized rats	IV	do	125	-	Anderson et al (1947) ¹
Alloxan-diabetic hypophysectomized adrenalectomized rats (ADHA rats)	IV	do	50	-	Bornstein (1950) ¹¹

IV Intravenous administration

II Intraperitoneal administration

SC Subcutaneous administration

cision (lambda) a statistical expression which includes an estimate both of the steepness of the dose effect curve, and of the deviation of the observations around the dose effect curve (87). A

low lambda value expresses a high degree of accuracy. It appears from table I that lambda for the *in vivo* methods is high, between 0.3 and 0.7. The strikingly low lambda in the study by Ar

IN VIVO METHODS

Brugsch & Horster published the first attempt at measuring the content of insulin in serum in 1930, when they examined an acid alcohol extract of serum by means of the mouse *convulsion test*, normally used for measuring the insulin content of pancreatic extracts. It was found, however, that intact mice could not be used for experiments to determine the insulin like effect of this extract (45). Hemmingsen & co-workers (119) showed that by removing the adrenals, the sensitivity of rats and mice to insulin could be increased. Gellhorn worked out a more sensitive technique, in which he used the decrease in blood sugar in hypophysectomized, adrenalectomized rats as an indicator of the insulin activity (91). This technique made it possible to demonstrate insulin-like activity in serum for the first time. Since then, other *in vivo* methods have been worked out, all of them based on the same principle: the insulin sensitivity of the animals is increased by removal of one or several of the endocrine organs which take part in the regulation of the blood sugar. Table 1 is a schematic presentation of these methods, their sensitivity and their accuracy.

Rafaelson (196) introduced an *in vivo* technique which was based on a new principle. He measured the changes in glycogen content in the diaphragm of the rat after an intraperitoneal injection of serum or standard insulin solutions into the intact animal. 100 μ L of insulin per ml solution was found to increase

the glycogen content, and in insulin like effect of serum could be determined by this method.

All the *in vivo* techniques are very difficult to work with on the whole (183, 203), and few studies using these methods have been published. The only methods which have proved sufficiently sensitive to register insulin like activity in serum are those of Gellhorn, Bornstein and Rafaelson. The other methods have been used mainly for determining the insulin like activity in protein concentrates or extracts from serum (2, 17, 25, 99).

Studies using *in vivo* techniques are of considerable interest, particularly when applied to measuring the insulin like activity of serum from the same animal species as the test animals. Under such circumstances, these studies will give the most accurate measurement of the physiological insulin like activity of the serum, i.e. corresponding to conditions in which insulin exerts its normal physiological activity.

Sensitivity

It appears from table 1 that the sensitivity of the *in vivo* methods varies considerably. The Bornstein method by which 50 μ U/ml can be determined, is the most sensitive method, while the other methods have sensitivities varying from 100 to 7000 μ U/ml.

Precision

The accuracy of biological methods is generally quoted as an index of pre-

method for determination of glycogen used in their study, however, the effect of insulin on the glycogen synthesis can only be demonstrated with insulin concentrations considerably higher than those which will increase the glucose uptake in rat diaphragm (194-244). Perlmutter & co-workers were thus unable to demonstrate ILA in serum by this technique. Recently, however, a method for determining insulin has been published which uses the synthesis of free glycogen in the rat diaphragm as a parameter for the insulin effect. This method seems to be sufficiently sensitive to determine SILA (128). Although it appears very promising, the method has not yet been taken up by other investigators.

Some studies use Stadler's "dipping technique" (246) for the examination of insulin antagonism in serum (see chapter 3). With this technique, the synthesis of glycogen is measured in rat diaphragm which has first been dipped for a short period in a solution of insulin, and then incubated in a buffer without insulin. This technique cannot be used for determining SILA but by comparing the glycogen content of two hemi diaphragms, one of which has been dipped in an insulin solution, the other in a mixture of serum and insulin solution it is possible to obtain a measure of the insulin inhibiting effect of serum.

A single study has been published in which the incorporation of C^{14} marked amino acids into rat diaphragm protein is used as a measure of the effect of the insulin. This method is sufficiently sen-

sitive to demonstrate insulin like activity in serum (164).

The rat diaphragm method has been used in a considerable number of studies on insulin like activity in serum. By far the majority of these investigations use the glucose uptake as a measure of the insulin effect. With this method, one part of a diaphragm is incubated in serum, and another part of the same diaphragm is incubated in a buffer without insulin. The difference between the uptake of glucose in the two incubation vessels, the "excess glucose uptake", can then be ascribed to the serum, and is used as a measure of the insulin like activity of the serum. On comparing the excess glucose uptake in the serum with the value measured by other diaphragms incubated in buffer containing a known insulin content, it is possible by interpolation to find an insulin concentration giving an excess glucose uptake equivalent to that of the serum. This concentration will then indicate the insulin like activity of the serum.

A mouse diaphragm method has been worked out which in principle corresponds in every way to the rat diaphragm method (180, 181, 291).

Table 2 shows the variants of the rat diaphragm method using glucose uptake as a measure of the insulin activity. In most of these investigations, the rat diaphragm is washed in ice-cold buffer immediately following removal, as a result, a reduced variation is obtained between the glucose uptakes in diaphragms from different animals (44). It appears from table 2 that most investigators have used

quilla & co-workers (15) is due to the fact that in this form of assay, a great number of animals are employed for measuring the insulin-like activity. A number of the studies cited do not give any information as to the precision of their methods.

Specificity

There are only a few studies in which the specificity of the *in vivo* methods is elucidated. Gellhorn & co-workers (92) found no ILA in serum from pancreatectomized dogs. Baird & Bornstein (17) showed with adrenalectomized, alloxan-diabetic mice that the insulin-like activity in an alcohol extract of serum disappears following treatment with cysteine, which is known to inactivate insulin. In studies with this method, the addition of insulin to the extract did not show any insulin-inhibiting factors in the extract.

Rafaelsen (197) used his method to examine the effect of hormones on the synthesis of glycogen in the rat diaphragm. Growth hormone in amounts of 10 $\mu\text{g}/\text{rat}$, glucagon (1 $\mu\text{g}/\text{rat}$) and desoxycorticosterone (10 $\mu\text{g}/\text{rat}$), increase the deposition of glycogen. Epinephrine (1 $\mu\text{g}/\text{rat}$) decreases the glycogen content of the rat diaphragm. Thus, these hormonal effects are found with amounts of hormone which are considerably greater than the amounts normally present in the volume of serum injected when Rafaelsen's method is used for determining SILA. Nevertheless, the possibility is not excluded

that even smaller amounts of hormone may influence SILA determinations by this method. It has not been proved, therefore, that those SILA values determined by Rafaelsen's method are independent of the hormones which have been studied. The fact that acetyl salicylic acid can increase the glycogen content of the rat diaphragm *in vivo*, may be of significance in the study of SILA *in vivo*.

THE RAT DIAPHRAGM METHOD

Gemmull was the first investigator to demonstrate the effect of insulin on glucose uptake and glycogen synthesis in the isolated rat diaphragm (93, 94, 95). These findings were subsequently confirmed by other investigators (136, 217, 244, 265, 285). Studies on the metabolism of the rat diaphragm have suggested that insulin stimulates the conversion of glucose to pyruvate and to carbon dioxide (284, 285) and shown that insulin stimulates the incorporation of amino acid in protein (137, 163, 238).

Groen & co-workers, in 1952, published a method for determining insulin like activity in serum using rat diaphragm as metabolically active tissue (103). These authors used glucose uptake as a measure of the insulin effect and showed that this technique could register the ILA in serum. At the same time Perlmutter & co-workers (186) attempted to measure the insulin like activity in serum by a rat diaphragm technique in which the synthesis of glycogen was used as an index of the insulin effect. With the

hemi-diaphragms, it is stated that further division of the diaphragm will result in a less pronounced *insulin response*, i.e. less difference between the glucose uptake in buffer with and without insulin (279). Some workers have used a 'pooling technique', i.e. portions of diaphragms from several animals are incubated in the same incubation vessel. As a result, a correction is obtained for any difference in the glucose uptake which may be present in diaphragms from different animals. Bicarbonate buffers were used to incubate the diaphragms in all methods except one, where a Krebs-Ringer phosphate buffer was used (259). Only a few workers have added a carrier protein (for instance gelatine) to the standard insulin solutions (63, 296). Such an addition will prevent the insulin in the solution from being adsorbed to the walls of the incubation vessels, whereby it is hindered in its effect on the diaphragm (121). Cunningham (63) could thus demonstrate that gelatine had a distinct influence on the effect of insulin on the glucose uptake of the diaphragm.

Comparing the glucose uptake in diaphragm samples incubated under similar test conditions the glucose uptake is considered in relation to the size of the piece of diaphragm. In most studies, the glucose uptake is calculated in relation to the weight of the diaphragm piece. Previously it was considered important for the accuracy of the experiments whether the glucose uptake had been calculated per g wet weight or per g dry weight. As the water content of the rat

diaphragm is fairly constant, however (161, 194, 279), this point is of no significance. The water content is known to be 75% of the total weight, so that a glucose uptake per g dry weight can be converted to a glucose uptake per g wet weight (Table 2).

In one investigation in which rats of almost identical weight were used, no correction was made for the diaphragm weight, the glucose uptake being calculated per hemidiaphragm (295).

As was anticipated, it appears from table 2 that the basal glucose uptake of the rat diaphragm, i.e. the uptake in a buffer without insulin, varies considerably in different studies. The excess glucose uptake resulting from a definite amount of insulin (in the table 100 μ U/ml) also varies considerably, viz. from 0.6 to 3.5 mg glucose/g diaphragm/90 minutes incubation time. This value varies in different rat strains and in animals of different size. A low excess glucose uptake, therefore, need not necessarily signify an unfortunate combination of the technical details mentioned above. It appears from table 2, however, that with a single exception, the sensitivity to insulin in the rat diaphragm method seems to be proportional to the excess glucose uptake produced by 100 μ U insulin per ml buffer.

Piazza & co-workers (192) showed that when the rat diaphragm method is used for measuring SIL_{125} , the amount of incubated tissue per incubation vessel is significant. Using I^{125} labelled insulin, these authors compared the insulin break-down in standard insulin solutions,

TABLE 2
Diaphragm methods

	Division of the diaphragm ¹⁾	Pieces of diaphragm per incubation vessel	Cool (mg ²⁾)	Time of incubation min.	Glucose concentration mg %	Basal glucose uptake mg g/90 min. ³⁾	Excess glucose uptake 100 μ l per ml min.	Sensitivity μ l. ml
<i>Rat diaphragm</i>								
Randle (1954) ^{100, 200} (1956) ¹⁰¹	1/2	6	—	180	250	7.0 (pr 180 min)	1.9 (pr 180 min)	100-2000
Groen et al (1952) ¹⁰²	1/2	4	+	90	200	7.6	2.0	10
Willebrands & Groen (1956) ¹⁰³	1/6	8	+	90	200		0.8	100
Tacheuchi et al (1957) ¹⁰⁴	1/2	4	+	90	200		1.1	1-10
Vallance Owen et al (1954) ¹⁰⁵ , Wright (1957) ¹⁰⁶ , Seltzer & Smith (1959) ¹⁰⁷	1/6	1	+	90	300	7.8	3.4	10
Staub (1958) ¹⁰⁸	1/3	1	+	120	200	4.5 (pr 120 min)	0.6 (pr 120 min)	100
Willebrands et al (1958) ¹⁰⁹	1/3	1	+	90	150	0.23 (pr hemidia phragm)	0.15 (pr hemidia phragm)	30
Metz (1960) ¹¹⁰	1/3	1	+	60	20-700		2.6	
Cunningham (1962) ⁸³	1/6	1	—	90	250	3.6	3.4	10
Jessup & Wiberg (1961) ¹¹¹	1/6	1	+	90	200	5.7	2.7	25
<i>Mouse diaphragm</i>								
Oyama & Grant (1960) ¹¹²	1/3	4	—	90	100	9.1	1.0	100
Wiberg & Jessup (1961) ¹¹¹	1/2	5	+	90	100	7.0	1.4 (produced by 20 μ l. ml)	10

¹⁾ 1/2 indicates that hemidiaphragms have been used

1/3 indicates that the hemidiaphragms have been cut into 5 pieces.

1/6 indicates that the hemidiaphragms have been cut into 8 pieces.

²⁾ Indicates whether the diaphragms have been washed in a cooled buffer before the incubation

³⁾ Calculated pr g wet weight (see pg. 19)

(see page 19) Where no carrier protein is present in these solutions, it may be assumed that part of the insulin will be adsorbed to the glassware. As a result, the excess glucose uptake measured in standard insulin solutions, will correspond to a lower insulin concentration than was intended. In agreement with this argument, it is found that the excess glucose uptake provoked by 100 μ U insulin per ml buffer, is higher in the variant of the rat diaphragm method in which gelatine is used in the standard insulin solutions (63), than it is in the great majority of the other variants (see table 2)

Sensitivity

Table 2 shows that the rat diaphragm methods which use glucose uptake as a measure of the insulin effect, vary considerably in sensitivity. A number of investigators report being able to demonstrate 10 μ U insulin per ml incubation medium whereas others only find an effect with insulin concentrations of 100 μ U/ml or more. More or less the same sensitivity seems to be obtained by the mouse diaphragm method as insulin concentrations from 10 to 100 μ U/ml can be demonstrated.

It is reported that 25 μ U/ml can be demonstrated by the rat diaphragm method, which uses glycogen deposition as a measure of the insulin effect (128), whereas 50 μ U/ml can be determined by the method of C^{14} glycine incorporation in diaphragm protein (164).

Precision

Studies in which the precision of the rat diaphragm method is calculated, indicate this value by the index of precision λ . Randle (202), in his modification of the method, found an average λ value of 0.34. Vallance-Owen (269) states that his modification shows λ values from 0.18 to 0.28, while other workers using the same modification find λ values from 0.20 to 0.55 (mean 0.28) (18). Takeuchi (259), using his own method, finds λ values of the same magnitude (mean 0.28), while the method which uses rat diaphragm divided into 8 pieces, shows a higher λ value (mean 0.51) (128).

λ values between 0.09 and 0.38 (mean 0.24) are found by the mouse diaphragm method (291). The same authors report that the rat diaphragm method, in which glycogen deposition is used as a measure of the insulin activity, has a λ value of 0.30 (128).

Specificity

A range of studies suggest that the rat diaphragm method measures an effect of insulin in serum. Most of the studies elucidating this problem have employed a modification of the method which measures SIAL by means of glucose uptake in the diaphragm. With this technique, it has been demonstrated that SIAL disappears following treatment of the serum with cysteine or glutathione, which are known to inactivate insulin.

in three variants of the rat diaphragm method. They found that the insulin break-down in standard insulin solutions was greatest with Randle's method, less with Groen's method and least with Vallance-Owen's method. This is in agreement with the finding that the insulin break-down is proportional to the number of incubated hemidiaphragms per ml incubation fluid.

On the basis of Piazza's investigations, therefore, it might be anticipated that in all modifications of the rat diaphragm method, but most pronounced in the variants with "pooling" techniques, the standard insulin solutions would give an excess glucose uptake which corresponded to a lower insulin concentration than intended. With one exception, the results obtained are in fact in accordance with those of Piazza. The excess glucose uptake elicited by 100 μ U insulin per ml buffer was found lowest in those variants of the rat diaphragm method in which several hemidiaphragms were used per incubation tube (see table 2).

Using the rat diaphragm method to determine the serum insulin-like activity, the excess glucose uptake due to the serum is related to the uptake elicited by insulin. In studies on insulin concentration and excess glucose uptake, different relationships were found between dose and response. In some studies a linear dependence was found between the cube root of the excess glucose uptake and the logarithm of the insulin concentration in the range 100–10,000 μ U/ml (199, 202, 294); other investigators found a linear dependence between the

excess glucose uptake and the square root of the insulin concentration in the range 10–1000 μ U/ml (272, 302). The majority of investigators found a linear dependence between the glucose uptake and the logarithm of the insulin concentration from 10–1000 μ U/ml (170, 235, 248, 291, 295, 259). Cunningham (63) found a linear dependence between the glucose uptake and the insulin concentration from 10 to 150 μ U/ml.

A linear dependence between the excess glucose uptake and the logarithm of the insulin concentration was found in the few studies in which the mouse diaphragm method was used (181, 291). Wardlaw & Moloney (287) described a mouse diaphragm method in which the insulin effect was measured by glycogen deposition in the diaphragm. Using this technique, and adding anti-insulin to the incubation vessel, an increase in the accuracy of the method was obtained at the expense of the sensitivity. These investigators found that over the range 10,000 μ U/ml to 20,000 μ U/ml there was a linear dependence between the glycogen synthesis, expressed as a percentage of the glycogen content of the diaphragm incubated in buffer without insulin, and the insulin concentration.

In using the rat diaphragm method to determine the serum insulin-like activity it is impossible to state with certainty which technical details are important and which are unimportant. It is reasonable, however, to attach considerable importance to the presence of a carrier protein in the standard insulin solutions.

(see page 19) Where no carrier protein is present in these solutions, it may be assumed that part of the insulin will be adsorbed to the glassware. As a result, the excess glucose uptake measured in standard insulin solutions, will correspond to a lower insulin concentration than was intended. In agreement with this argument, it is found that the excess glucose uptake provoked by 100 μ U insulin per ml buffer, is higher in the variant of the rat diaphragm method in which gelatine is used in the standard insulin solutions (63), than it is in the great majority of the other variants (see table 2)

Sensitivity

Table 2 shows that the rat diaphragm methods which use glucose uptake as a measure of the insulin effect, vary considerably in sensitivity. A number of investigators report being able to demonstrate 10 μ U insulin per ml incubation medium whereas others only find an effect with insulin concentrations of 100 μ U/ml or more. More or less the same sensitivity seems to be obtained by the mouse diaphragm method, as insulin concentrations from 10 to 100 μ U/ml can be demonstrated.

It is reported that 25 μ U/ml can be demonstrated by the rat diaphragm method, which uses glycogen deposition as a measure of the insulin effect (128), whereas 50 μ U/ml can be determined by the method of C^{14} glycine incorporation in diaphragm protein (164).

Precision

Studies in which the precision of the rat diaphragm method is calculated, indicate this value by the index of precision "lambda". Randle (202), in his modification of the method, found an average lambda value of 0.34. Vallance-Owen (269) states that his modification shows lambda values from 0.18 to 0.28, while other workers using the same modification find lambda values from 0.20 to 0.55 (mean 0.28) (18). Takeuchi (259), using his own method, finds lambda values of the same magnitude (mean 0.28), while the method which uses rat diaphragm divided into 8 pieces, shows a higher lambda value (mean 0.51) (128).

Lambda values between 0.09 and 0.38 (mean 0.24) are found by the mouse diaphragm method (291). The same authors report that the rat diaphragm method in which glycogen deposition is used as a measure of the insulin activity, has a lambda value of 0.30 (128).

Specificity

A range of studies suggest that the rat diaphragm method measures an effect of insulin in serum. Most of the studies elucidating this problem have employed a modification of the method which measures SILA by means of glucose uptake in the diaphragm. With this technique, it has been demonstrated that SILA disappears following treatment of the serum with cysteine or glutathione, which are known to inactivate insulin.

(105, 199, 271) Other investigators have demonstrated that serum to which anti-insulin has been added neither stimulates glucose uptake nor effects the incorporation of C^{14} -glycine into diaphragm protein (164, 274, 303) In dogs, Metz (170) found a higher SILA in blood from the pancreatoduodenal vein than in blood from the femoral artery SILA cannot be demonstrated by the rat diaphragm method in pancreatectomized cats (275), and in dogs which have been pancreatectomized, a steady fall in SILA sets in following the pancreatectomy, decreasing gradually for 20 days until less than 10% of the initial value (105)

These investigations seem to indicate that when measured by the rat diaphragm method, SILA is due to insulin It is an open question, however, whether the measurement of SILA by this technique indicates solely the amount of biologically active insulin in serum, or whether serum contains factors of perhaps hormonal character, which influence the SILA determinations by inhibiting or stimulating the activity of insulin Table 3 lists investigations of the *in vitro* effect of different hormones on glucose uptake and glycogen synthesis in isolated rat diaphragm from normal animals The table shows that in a few studies, growth hormone was found to have an insulin-like effect on this tissue, but as this effect can only be demonstrated in a phosphate buffer and not in a bicarbonate buffer (207), growth hormone is unlikely to influence the determination of SILA by the rat

diaphragm method Table 3 shows that a variety of hormones inhibit both the glucose uptake and the glycogen synthesis in rat diaphragm The inhibiting effect of epinephrine on these metabolic processes is found at epinephrine concentrations in the incubation medium which correspond to the concentrations normally found in serum Norepinephrine in physiological concentrations likewise inhibits glucose uptake in rat diaphragm On the other hand, the inhibiting effect of cortisol, corticosterone, desoxycorticosterone and glucagon on the metabolic processes in rat diaphragm is demonstrated at hormone concentrations which are considerably higher than physiological levels The stimulation of C^{14} -glycine incorporation by ACTH has likewise been demonstrated at concentrations far higher than the concentrations in serum (163) These investigations, however, do not exclude the possibility that the hormones mentioned may have an effect on the metabolic processes in rat diaphragm even in physiological concentrations

The cited studies of the effect of norepinephrine and epinephrine on the glucose metabolism of the rat diaphragm suggest that the presence of these hormones in serum can inhibit the effect of insulin, and that measured by the rat diaphragm method therefore SILA can hardly be a quantitative indication of the amount of biologically active insulin in serum As it is possible, however, to recover quantitatively the amount of insulin which has been added to undiluted serum (170, 199, 271), it must be sup-

TABLE 3

The in vitro effect of hormones on the glucose metabolism in rat diaphragm

	The hormone concentration in the serum of normal subjects (mean value or range)	Glucose uptake	Glucose synthesis
Growth hormone	200 (80-300) $\mu\text{g}/\text{ml}$ Ehrlich & Randle (1961) ¹²⁵ 50-100 $\mu\text{g}/\text{ml}$ Read & Bryan (1960) ¹²⁶ 170 (90-250) $\mu\text{g}/\text{ml}$ Hartog & Fraser (1961) ¹¹⁷ 5 $\mu\text{g}/\text{ml}$ McGarry et al (1960) ¹²⁰ 0-50 $\mu\text{g}/\text{ml}$ Unger et al (1962) ¹²²	+ 10 $\mu\text{g}/\text{ml}$ Ottaway (1953) ¹¹⁷ + 1 $\mu\text{g}/\text{ml}$ Ottaway (1961) ¹²⁸ + 25 $\mu\text{g}/\text{ml}$ Randle & Whitney (1957) ¹⁰⁹ 0 100 $\mu\text{g}/\text{ml}$ Randle & Young (1956) ¹⁰⁰ 0 10-100 $\mu\text{g}/\text{ml}$ Park et al (1952) ¹⁰² 0 50 $\mu\text{g}/\text{ml}$ Manchester & Young (1959) ¹⁴²	0 500 $\mu\text{g}/\text{ml}$ Stadie et al (1949) ¹¹⁶
ACTH	0.1 mU/100 ml Lupton (1957) ¹¹⁸ 1.0 mU/100 ml Bethune et al (1958) ¹¹⁹ 200 mU/100 ml Bornstein & Trowhella (1950) ¹²⁰ 160 mU/100 ml Montanari et al (1951) ¹¹⁹ 35 mU/100 ml Martinelli & Montanari (1955) ¹¹⁹ 110-170 mU/100 ml Ceresa & Reynes (1953) ¹²¹	0 200 $\mu\text{g}/\text{ml}$ Ottaway & Buisbrook (1953) ¹¹⁸ 0 25 $\mu\text{g}/\text{ml}$ Randle & Young (1957) ¹⁰⁹	
Cortisol	5-25 $\mu\text{g}/100\text{ ml}$ Elk Weiss (1960) ¹²⁴		0 50 $\mu\text{g}/\text{ml}$ Stadie et al (1957) ¹¹⁶
Corticosterone	2-6 $\mu\text{g}/100\text{ ml}$ Elk Weiss (1960) ¹²⁴	0 10 $\mu\text{g}/\text{ml}$ Bartlett & Wick (1949) ¹²³	0 10 $\mu\text{g}/\text{ml}$ Bartlett & Wick (1949) ¹²³
Desoxycorticosterone		0 10 $\mu\text{g}/\text{ml}$ Bartlett & Wick (1949) ¹²³ — 40 $\mu\text{g}/\text{ml}$ Leupin & Verzar (1949) ¹²²	0 10 $\mu\text{g}/\text{ml}$ Bartlett & Wick (1949) ¹²³ — 40 $\mu\text{g}/\text{ml}$ Leupin & Verzar (1949) ¹²²

TABLE 3 (continued)

	The hormone concentration in the serum of normal subjects (mean value or range)	Glucose uptake	Glucose synthesis
Epinephrine	0.6 $\mu\text{g}/100 \text{ ml}$ Cohen & Goldenberg (1957) ¹⁰⁰ 0.0 $\mu\text{g}/100 \text{ ml}$ Valk & Price (1956) ¹⁰¹ 2.5 $\mu\text{g}/100 \text{ ml}$ Weil, Malherbe & Bone (1951) ¹⁰² 1.1 $\mu\text{g}/100 \text{ ml}$ Weil, Malherbe & Bone (1957) ¹⁰³ 2.4 $\mu\text{g}/100 \text{ ml}$ Griswold (1958) ¹⁰⁴	- 0.20 $\mu\text{g}/\text{ml}$ Waldes & Waldes (1956) ¹⁰⁵ - 0.01 $\mu\text{g}/100 \text{ ml}$ Groen et al (1958) ¹⁰⁶	- 0.25 $\mu\text{g}/\text{ml}$ Waldes & Waldes (1956) ¹⁰⁵ - 0.33 $\mu\text{g}/\text{ml}$ Juerkischer & Wettheimer (1948) ¹⁰⁷ - 0.5 $\mu\text{g}/\text{ml}$ Riesser (1947) ¹⁰⁸
Norepinephrine	3 $\mu\text{g}/100 \text{ ml}$ Cohen & Goldenberg (1957) ¹⁰⁰ 2 $\mu\text{g}/100 \text{ ml}$ Valk & Price (1956) ¹⁰¹ 4.1 $\mu\text{g}/100 \text{ ml}$ Weil, Malherbe & Bone (1957) ¹⁰³ 5.7 $\mu\text{g}/100 \text{ ml}$ Weil, Malherbe & Bone (1951) ¹⁰² 32 $\mu\text{g}/100 \text{ ml}$ Griswold (1958) ¹⁰⁴	- 0.75 $\mu\text{g}/\text{ml}$ Waldes & Waldes (1956) ¹⁰⁵ - 0.05 $\mu\text{g}/\text{ml}$ Groen et al (1958) ¹⁰⁶	1.0 $\mu\text{g}/\text{ml}$ Waldes & Waldes (1956) ¹⁰⁵
Glucagon	350 (0-500) $\mu\text{g}/\text{ml}$ Unger et al (1962) ¹⁰⁹	50 $\mu\text{g}/\text{ml}$ Landells (1953) ¹¹⁰	100 $\mu\text{g}/\text{ml}$ Smedley et al (1955) ¹¹¹

4 indicates that a stimulation has been registered

- indicates that an inhibition has been registered

0 indicates that no effect has been registered

posed that the presumed epinephrine/norepinephrine inhibition in undiluted serum has been compensated by the insulin present.

Several investigations suggest that other substances than insulin are able to increase the glucose uptake and the glycogen synthesis in rat diaphragm. Ca

strillon & co-workers (5) have thus briefly reported that in a concentration corresponding to the physiological level (2 mg/g (250)) leucine will increase both these metabolic processes. Hvalby & Walis (12), in a detailed study, found an ultrafiltrable serum factor which stimulated the glucose uptake in

rat diaphragm. The glucose uptake is likewise increased by carbutamide and tolbutamide (195), phenethylguanide (57, 195, 296), sodium salicylate (162) and a number of enzyme poisons such as 2,4-dinitrophenol, sodium arsenate and sodium cyanide (208).

THE RAT EPIDIDYMAL FAT METHOD

Rat epididymal fat was used for the first time in metabolic studies in 1954 (118). Later investigations showed that insulin increased epididymal fat's glucose uptake and oxidation of glucose to carbon dioxide *in vitro* (126, 297). These findings have later been confirmed in numerous studies (53, 62, 69, 111, 112, 141, 146, 152, 305). It has further been demonstrated that insulin stimulates the rat epididymal fat's conversion of glucose to lactate (112) to glycogen (49, 146) and to fatty acids (49, 146, 298).

Renold & co-workers (166) worked out the method for determining the insulin-like activity of serum by using rat epididymal fat as a metabolically active tissue. At the same time, v. der Geld, Willebrands & Groen (90) published a similar technique with rat mesenteric fat. This latter technique has not been rechecked, whereas Renold's rat epididymal fat method has been used in a great number of investigations. In the study cited, Renold used the oxidation of glucose 1- C^{14} to $C^{14}O$ as a measure of the insulin effect, but it is possible to use all the above-mentioned insulin-susceptible metabolic parameters to determine the insulin-like activity in serum. All the major studies so far published

on SILA use the fatty tissue's glucose uptake, oxidation of glucose 1- C^{14} to $C^{14}O_2$, and carbon dioxide production, to measure SILA.

The method whereby SILA is measured by the carbon dioxide production, utilizes the fact that insulin provokes an increase in the respiratory quotient of fatty tissue, and of the organism as a whole. This increase is due to an increase in the carbon dioxide production, whereas the oxygen uptake only shows small and uncertain variations. It has therefore been found permissible to measure the resulting increase in pressure, the 'net gas exchange', in a Warburg apparatus as expressing the increase in the respiratory quotient.

In the modifications of the rat epididymal fat method mentioned, advantage is taken of the fact that it is possible to establish a linear dependence between the insulin concentration in the incubation medium and the metabolic response of the fatty tissue to insulin. In calculating this relation, allowance must be made for the fact that the weight of incubated fatty tissue is not the same in all incubation vessels. A large fat sample will have a greater glucose metabolism than a small fat sample, even under otherwise constant conditions. Renold (166) made a correction for this by calculating the amount of $C^{14}O_2$ developed per g fatty tissue. Other investigators, using smaller fat samples, found that the ratio

$$\frac{\text{amount of } C^{14}O_2 \text{ developed}}{\sqrt{\text{fat weight}}}$$

TABLE 3 (continued)

	The hormone concentration in the serum of normal subjects (mean value or range)	Glucose uptake	Glucagon synthesis
Epinephrine	0.6 $\mu\text{g}/100$ ml Cohen & Goldenberg (1957) ¹⁰⁰	- 0.20 $\mu\text{g}/\text{ml}$ Wallés & Wallés (1956) ¹⁰⁰	- 0.25 $\mu\text{g}/\text{ml}$ Wallés & Wallés (1956) ¹⁰⁰
	0.0 $\mu\text{g}/100$ ml Valk & Price (1956) ¹⁰⁰	- 0.01 $\mu\text{g}/100$ ml Groen et al (1958) ¹⁰⁰	- 0.33 $\mu\text{g}/\text{ml}$ Guertlicher & Wertheimer (1948) ¹⁰⁰
	2.5 $\mu\text{g}/100$ ml Weil Malherbe & Bone (1951) ¹⁰⁰		- 5 $\mu\text{g}/\text{ml}$ Riesser (1947) ¹⁰⁰
	11 $\mu\text{g}/100$ ml Weil Malherbe & Bone (1957) ¹⁰⁰		
	2.4 $\mu\text{g}/100$ ml Griswold (1958) ¹⁰¹		
Norepinephrine	3 $\mu\text{g}/100$ ml Cohen & Goldenberg (1957) ¹⁰⁰	- 0.75 $\mu\text{g}/\text{ml}$ Wallés & Wallés (1956) ¹⁰⁰	- 1.0 $\mu\text{g}/\text{ml}$ Wallés & Wallés (1956) ¹⁰⁰
	2 $\mu\text{g}/100$ ml Valk & Price (1956) ¹⁰⁰	- 0.03 $\mu\text{g}/\text{ml}$ Groen et al (1958) ¹⁰⁰	
	11 $\mu\text{g}/100$ ml Weil Malherbe & Bone (1957) ¹⁰⁰		
	57 $\mu\text{g}/100$ ml Weil Malherbe & Bone (1951) ¹⁰⁰		
	32 $\mu\text{g}/100$ ml Griswold (1958) ¹⁰¹		
Glucagon	3.0 (0-900) $\mu\text{g}/\text{ml}$ Unzer et al (1962) ¹⁰⁰	- 50 $\mu\text{g}/\text{ml}$ Candela (1953) ¹⁰¹	- 100 $\mu\text{g}/\text{ml}$ Smedecur et al (1955) ¹⁰¹

+ indicates that a stimulation has been registered

- indicates that an inhibition has been registered

|| indicates that no effect has been registered

posed that the presumed epinephrine-norepinephrine inhibition in undiluted serum has been compensated by the insulin present.

Several investigations suggest that other substances than insulin are able to increase the glucose uptake and the glycogen synthesis in rat diaphragm. Ca-

strillon & co-workers⁵³ have thus briefly reported that in a concentration corresponding to the physiological level (2 mg%, 250) leucine will increase both these metabolic processes. Hvalby & Wilas¹²⁵ in a detailed study found an ultrafiltrable serum factor which stimulated the glucose uptake in

been found in some minor test series, i.e. lower lambda values Steelman (249), for example, found constant values less than 0.15, and in other studies the mean values for lambda are given as 0.18 (290) and 0.12 (47). Using the 'net gas exchange' method, Burgi & co-workers found the same lambda values. The greater accuracy of the latter over the former studies is hardly due to a different metabolic parameter being used for measuring the insulin effect. In the more accurate investigations, however, it is decisive that a greater number of rats were used per assay than in the other studies.

Specificity

It has often been discussed whether the rat epididymal fat method, in addition to insulin, measures other factors in serum which have the same effect on the glucose metabolism of the fatty tissue as insulin (30, 145). This is a conclusion which suggests itself for several reasons. For one thing, it has been established that SILA measured by the rat epididymal fat method does not disappear following pancreatectomy whereas insulin measured immunologically, and SILA measured by the rat diaphragm method, both disappear. For another thing, SILA measured by the rat epididymal fat method is only partially inhibited after addition of anti-insulin; this also in contrast to experience with the rat diaphragm method. In addition, several hormones and amino acids such as leucine and glutathione are present in se-

rum, and with respect to some parameters, their effect on the glucose metabolism of the rat epididymal fat corresponds to that of insulin.

Using the rat epididymal fat method, a number of investigators (74, 145, 146) have been able to demonstrate SILA in apparently totally pancreatectomized experimental animals, in Goldberg & Eg-dahl's investigation, in spite of the fact that insulin could not be demonstrated in serum by the immunological method. These findings have been adduced as an argument that in addition to insulin, the rat epididymal fat method measures other factors in serum. The argument, however, is hardly valid. Using the rat epididymal fat method, it was thus shown that SILA disappeared in pancreatectomized animals following treatment of serum with glutathione (257). The same investigators also found that 5 days after pancreatectomy in dogs, extraction of the serum with acid alcohol gave an insulin-like activity in the extract, and part of this activity could be inhibited by anti-insulin. This investigation would appear to demonstrate unambiguously that insulin is found in serum some time after 'total' pancreatectomy. The reason for this has not been elucidated, but investigations by Samaan & co-workers (229) are of considerable interest in this connection. These workers found that apparently totally pancreatectomized dogs presented a pronounced hypoglycaemic reaction following injection of alloxane. They claimed that this phenomenon supported the theory that ectopic pancreatic tissue,

gives a better correction for variations in the fat weights (152, 240). In agreement with this, both Renold (216) and Beigelman (24) found that the glucose metabolism per g of fatty tissue is higher for fat pieces less than 70 mg than it is for larger pieces.

In their first studies, Renold & co-workers found a linear relationship between the logarithm of the insulin concentration in the range 30 to 500 μ U/ml and the square root of the amount of $C^{14}O_2$ developed/g fatty tissue (166). In a subsequent study, however, they showed that by using the relation between the logarithm of the amount of $C^{14}O_2$ developed/g fatty tissue and the logarithm of the insulin concentration, there was less variation in the SILA determinations (lower lambda) (216). This linear dependence between log dose and log response was confirmed by later investigators (146, 152, 189, 226, 290).

Several investigators used the glucose uptake of the epididymal fat as a measure of the insulin effect, and found a linear dependence between the glucose uptake per g fatty tissue and the logarithm of the insulin concentration (123, 198, 248, 249, 290). Others, again, found a linear dependence between the logarithm of the glucose uptake per g tissue and the logarithm of the insulin concentration (24, 146).

In some studies, the "net gas exchange" of the epididymal fat is used as a measure of the insulin effect. Ramsey et al (198) found a linear dependence between the gas evolved per g fat tis-

sue per hour and the logarithm of the insulin concentration in the range 10-1000 μ U/ml. Another variant of this method calculates the relation between the evolution of gas resulting from incubation of a sample of fat with a solution containing unknown ILA and the evolution of gas in the same incubation vessel, when a very large amount of insulin (0.1 U/ml) is added to the solution, after this has been incubated for some time. Between this relation, the "percentage of maximal response", and the insulin concentration, there is a linear dependence in the range 10-100 μ U insulin per ml (19, 140).

Sensitivity

The three modifications of the rat epididymal fat method—the use of glucose uptake, the "net gas exchange", and the oxidation of glucose-1 C^{14} , all seem to have more or less the same sensitivity. They are all able to measure insulin amounts of 10 μ U per ml incubation liquid (23, 111, 167, 240, 300).

Precision

In many determinations using the oxidation of glucose-1- C^{14} as a measure of the insulin effect, Renold's group find a mean lambda of 0.30 (237). Other investigators, using the same procedure, find mean lambda values of 0.28 (70, 152) and 0.22 (290).

Where the glucose uptake of the rat epididymal fat is used as a measure of the insulin effect, a greater accuracy has

TABLE 4

The in vivo effects of hormones on the glucose metabolism in rat epididymal fat

	Hormone concentration (from normal subjects)	Glucose	Glucose	% gas exchange	Glucose-1-C
Growth hormone	0 mg/g 5 g/ml	+ 100 g/ml	Lebeuf & Cahill (1961) ¹²	0 1 mg/ml Jungas & Ball (1960) ^{12b}	+ 200 g/ml W negrad et al (1959) ^{12a}
		+ 1 mg/ml	Jungas & Ball (1960) ^{12b}		+ 1 mg/ml Lebeuf & Cahill (1961) ¹²
		0 100 g/ml	D tschune t et al (1961) ¹		0 100 g/ml D tschune t et al (1961) ¹
ACTH	0 1 200 mU/ 100 ml	+ 100 g/ml	Lebeuf & Cahill (1961) ¹²		+ 20 g/ml Lynn et al (1960) ^{12a}
					0 100 g/ml Lebeuf & Cahill (1961) ¹²
Cortisol	5-25 g/ 100 ml	+ 0 1 µg/ml	D tschune t et al (1961) ¹		0 30 g/ml Jeanrenaud & Renold (1960) ^{12c}
		0 30 g/ml	Lebeuf et al (1962) ¹²		0 10 g/ml D tschune t et al (1961) ¹
Epinephrine	0 2 g/ 100 l	+ 18 g/ml	Lebeuf et al (1959 1961) ^{12, 12a}	+ 10 g/ml Hagen & Ball (1960) ^{12a}	+ 20 µg/ml Lynn et al (1960) ^{12a}
		10 g/ml	Hagen & Ball (1960) ¹²	+ 1 g/ml Correa & Magelhaes (1961) ¹²	+ 18 µg/ml Cahill et al (1960) ¹²
		0 001 g/ml	Humbel (1959) ¹²		
Glucagon	0 300 mean 3.0) g/ml	+ 0 02 g/ml	D tschune t et al (1961) ¹²		0 1 g/ml D tschune t et al (1961) ¹²
		+ 0 01 g/ml	Hagen (1961) ¹²		+ 0 4 g/ml Wörner & Weinges (1961) ^{12a}
		+ 100 g/ml	Lee et al (1960) ^{12a}		
		+ 40 g/ml	Wörner & Weinges (1961) ^{12a}		

For references see table 3

which had not been removed by the operation, must be present in the dogs. The reason why the insulin in serum from pancreatectomized dogs cannot be demonstrated by the immunological method or by the rat diaphragm method, is presumably either that the insulin is present in a state in which it has no effect on the two test systems, or that with respect to the rat diaphragm method, its effect is inhibited by insulin antagonists (275).

In several studies on the rat epididymal fat method, SILA was examined after the addition of anti insulin to serum. Between 5 and 30 % of the SILA was found to be inhibited by anti-insulin (47, 146, 159, 198, 226, 227). This was taken as a proof of the hypothesis that in addition to insulin, serum contains other factors with insulin-like activity on rat epididymal fat. It has been shown, however, that electrophoretically-separated serum protein fractions contain significant amounts of insulin in an immunologically inactive state (156). It is probable that the SILA fraction which is not inhibited by anti insulin in the investigations with the rat epididymal fat method, is due to this immunologically inactive insulin.

A number of investigations support the assumption that the insulin-like activity which can be registered by the rat epididymal fat method, is due to insulin. For example, it has been demonstrated that SILA measured by the rat epididymal fat method disappears following treatment of serum with cysteine or glutathione, which inactivate insulin (216,

226). It has been shown that SILA is higher in pancreatic venous blood than in peripheral venous blood (167, 187, 226), and that there is a rise in SILA following glucose administration in normal subjects (48, 187, 153, 190, 226, 227). The addition of insulin to undiluted serum is followed by a rise in SILA, corresponding to the amount of insulin added (96, 123, 155, 187).

In order to further characterize the ILA in serum, two investigators have examined the simultaneous action of serum on a number of metabolic processes in the fat. Ramsey et al (198) examined the effect of serum on the following processes in rat epididymal fat: glucose uptake, net gas exchange, oxidation of uniformly marked glucose C^{14} to $C^{14}O$ and incorporation of C^{14} in fatty acids. With these parameters, identical SILA values were found on studying serum diluted five fold. Leonards et al (146) examined SILA in a corresponding manner by means of the following parameters: glucose uptake, conversion of glucose-1- C^{14} to $C^{14}O$, glycogen, fatty acids and glycerol. This investigation showed identical values for SILA with five of the parameters mentioned; only the method with incorporation in glycogen resulted in lower values than the other tests. Leonards took this result to support the assumption that glycogen synthesis from glucose is a more specific measure of the insulin activity than the other metabolic parameters examined. Renold (213), who made extensive determinations of C^{14} incorporation in rat epididymal fat glycogen has suggested

TABLE 4

The in vitro effects of hormones on the glucose metabolism in rat epididymal fat

Hormone concentration in serum (from normal subjects)		Glucose uptake		Net exchange		Glucose-1-C-14 CO ₂	
Growth hormone	0.1 mg/ml	+ 100 µg/ml	Lebeuf & Cahill (1961) ¹²²	0.1 mg/ml	Jungas & Ball (1960) ¹²³	+ 200 µg/ml	Winegrad et al. (1959) ¹²⁴
	0.1 mg/ml	+ 1 mg/ml	Jungas & Ball (1960) ¹²³			+ 1 mg/ml	Lebeuf & Cahill (1961) ¹²²
	0.1 mg/ml	0.100 µg/ml	Ditschuneit et al. (1961) ⁷¹			0.100 µg/ml	Ditschuneit et al. (1961) ⁷¹
ACTH	0.1-200 mU/100 ml	+ 100 µg/ml	Lebeuf & Cahill (1961) ¹²²			+ 20 µg/ml	Lynn et al. (1960) ¹²¹
		0.100 µg/ml	Lebeuf et al. (1962) ¹²⁵			0.100 µg/ml	Lebeuf & Cahill (1961) ¹²²
Cortisol	0.25 µg/100 ml	+ 0.1 µg/ml	Ditschuneit et al. (1961) ⁷¹			0.30 µg/ml	Jeanrenaud & Renold (1960) ¹²⁷
		0.30 µg/ml	Lebeuf et al. (1962) ¹²⁵			0.10 µg/ml	Ditschuneit et al. (1961) ⁷¹
Epinephrine	0.2-2 µg/100 ml	+ 18 µg/ml	Lebeuf et al. (1959, 1961) ^{121, 122}	+ 10 µg/ml	Hagen & Ball (1960) ¹¹²	+ 20 µg/ml	Lynn et al. (1960) ¹²¹
		+ 10 µg/ml	Hagen & Ball (1960) ¹¹²	+ 1 µg/ml	Correa & Magelhaes (1961) ¹²⁸	+ 18 µg/ml	Cahill et al. (1960) ⁴⁸
		0.001 µg/ml	Humbel (1959) ¹²⁹				
Glucagon	0-900 (mean 350) µg/nl	+ 0.02 µg/ml	Ditschuneit et al. (1961) ⁷¹			0.1 µg/ml	Ditschuneit et al. (1961) ⁷¹
		+ 0.01 µg/ml	Hagen (1961) ¹¹²			+ 0.4 µg/ml	Werner & Weinges (1961) ¹²⁶
		+ 100 µg/ml	Lee et al. (1960) ¹²⁴				
		+ 40 µg/ml	Werner & Weinges (1961) ¹²⁶				

¹ For references see table 3

that the lower SILA values which Leonards found by means of this technique, might be due to technical problems in connection with the extraction of the very small amounts of glycogen present in fatty tissue, and with the determination of the C^{14} content of the extract.

A number of organic compounds have been shown to possess an insulin like effect on the glucose metabolism in rat epididymal fat. Table 4 is a schematic representation of those studies made on hormonal *in vitro* effects on metabolic processes in rat epididymal fat employed in methods for determining the insulin-like activity in serum. It appears from the table that the glucose uptake in rat epididymal fat is stimulated by growth hormone, ACTH, epinephrine and glucagon in concentrations which are markedly higher than the physiological levels. The "net gas exchange" of fatty tissue is stimulated by epinephrine in concentrations which are higher than the physiological levels. The oxidation of glucose-1- C^{14} to $C^{14}O_2$ is stimulated by growth hormone and epinephrine, possibly also by ACTH and glucagon, but studies on these hormones give contradictory results. A single study demonstrated that all metabolic parameters were stimulated by prolactin in concentrations of 50-100 mg% (299). Thus, even though these hormonal effects on the glucose metabolism of the rat epididymal fat have all been demonstrated at hormonal concentrations significantly higher than the physiological levels, this of course does not exclude the hormones having an insulin like effect also in phy-

siological concentrations. It is seen from table 4, however, that only few studies have been made on the effect of physiological concentrations of hormone on the glucose metabolism of rat epididymal fat.

Castrillon & co-workers (35) have reported that leucine in a concentration of 2 mg%, corresponding to the physiological level of leucine in plasma, is able to increase glucose uptake and glycogen synthesis by rat epididymal fat. The study cited does not permit a more exact evaluation of the technique used. In particular, it is not possible to decide whether allowance has been made for the fact that fatty tissue from different sites in the lump of epididymal fat, varies in glucose uptake.

In a study of the effect of leucine (2 mg%) on the oxidation of glucose-1- C^{14} to $C^{14}O_2$, and in which attention was paid to the above factor, no effect of leucine on this process could be found (158).

When present in high concentrations a number of organic substances occurring in the living organism have an insulin like effect on the oxidation of glucose-1- C^{14} to $C^{14}O_2$ in rat epididymal fat. For example, reduced glutathione in a concentration of 30 mM (216) ribonucleic acid, adenylic acid and adenosine in a concentration of 100 mg% (73) and oxytocin (100 ml/ml) (171). It is of considerable theoretical interest that also the phenylalanyl chain of insulin is able to accelerate the glucose metabolism in fatty tissue (139).

The effect of peroral anti diabetic

drugs on rat epididymal fat was examined in a few studies. Tolbutamide and chlorpropanide stimulate the oxidation of glucose 1 C¹⁴ to C¹⁴O (215). It is stated that tolbutamide stimulates both glucose uptake and glycogen synthesis in rat epididymal fat (54), but this was not confirmed elsewhere (70). In the latter study, on the other hand, it was found that phenylethyl biguanide increased glucose uptake in rat epididymal fat.

IMMUNOLOGICAL METHODS

Arquilla & Stavitsky (13) were the first to employ immunological principles for the determination of small amounts of insulin. They used a haemagglutination-inhibition technique with insulin antibody from immunized rabbits. This method was not sufficiently sensitive to demonstrate insulin in serum. It has been used during recent years, however, for immunological determination of insulin in serum extracts (184-222).

Yalow & Berson (307) have worked out an immunological method for determining insulin based on a completely new principle. They had found that dissolved insulin labelled with I¹³¹ is bound to insulin antibody. In solutions with different concentrations of non radioactive insulin and a fixed concentration of I¹³¹ labelled insulin, the amount of antibody bound I¹³¹ labelled insulin will depend on the amount of non radioactive insulin in the solution. A high ratio between radioactive and non radioactive insulin will result in a large amount

of I¹³¹ labelled insulin being bound to antibody, and a low ratio, a low amount. After separation of free and antibody-bound I¹³¹-labelled insulin, the amount of antibody bound I¹³¹ labelled insulin can be measured immediately by means of its radioactivity. Berson & Yalow found that this separation was possible by means of paper electrophoresis. They showed that in paper electrophoresis, when serum without insulin antibody is added to I¹³¹-labelled insulin, the labelled insulin is adsorbed at the site of application (29). In electrophoresis of a mixture of I¹³¹ labelled insulin and insulin antibody, the bound insulin migrates with the antibody. If electrophoresis is performed on a solution containing a mixture of I¹³¹ labelled insulin bound to anti insulin and free I¹³¹-labelled insulin, two maxima will be found for the radioactivity, one at the site of application, corresponding to the free I¹³¹ labelled insulin, and one in the γ globulin range, corresponding to the antibody bound insulin. The ratio between the radioactive radiation in these two regions corresponds to the ratio between free and antibody bound I¹³¹-labelled insulin. To measure the insulin content of a solution, the solution is mixed with I¹³¹ labelled insulin, anti insulin is added, and electrophoresis is then carried out on the solution, the ratio between free and antibody bound I¹³¹-labelled insulin being measured. On comparison with the ratio obtained between free and antibody bound I¹³¹-labelled insulin when using standard insulin solutions, a measure will be ob-

tained for the content of immunologically active insulin in the "unknown solution"

Yalow & Berson's method is so sensitive that it can demonstrate insulin in serum. It has been used by other investigators, who were able to reproduce Berson & Yalow's results (230).

After the publication of Yalow & Berson's method, other immunological methods appeared, which used the same principle, but in which the free and bound I^{131} -labelled insulin were separated by other means. Grodsky & Forsham (103) worked out a method in which the separation is by precipitation of the bound I^{131} -labelled insulin with sodium sulphite. This method has been used for determination of insulin in serum extracts made with acid alcohol. With this technique, insulin could not be demonstrated in fasting serum, but on the other hand insulin could be demonstrated in a serum sample drawn 30 minutes after administration of glucose. There is thus no doubt that Grodsky & Forsham's method is less sensitive than that of Yalow & Berson, though the reason is not clear. It is possible that the relatively low sensitivity in Grodsky & Forsham's method may be due to the fact that the sodium sulphite precipitation results in a poorer separation of free and antibody-bound I^{131} -labelled insulin than is obtained by paper electrophoresis, but the data published do not permit any conclusion to be drawn.

Already in 1951 however Skom & Lalmage had established that the insulin anti insulin complex in serum could be

precipitated if anti globulin was also added (239). Various investigators have used this method for the separation of free and antibody-bound I^{131} labelled insulin (100, 113, 174). Insulin in fasting serum from normal subjects has been determined by these methods.

Unless special precautions are taken, Yalow & Berson's immunological method, or methods based on the same principle, cannot be used for determining the insulin in serum from patients who have had insulin treatment. This is due to the fact that insulin antibody can be demonstrated in human serum after a certain period of insulin treatment. This antibody will influence the insulin-anti insulin reaction in the test system. By ultracentrifugation of serum however, it is possible to sediment the insulin antibody and to determine the antibody free supernatant. The amount of immunologically-determined insulin in the supernatant will then constitute a measure of the insulin content of the serum. Berson & Yalow have further shown that by making high dilutions of serum, it is possible to eliminate the effect of the anti insulin. A measurement of the immunologically-determined insulin in highly diluted serum makes it possible to calculate the content in undiluted serum. Berson & Yalow (31) have shown that these two methods give identical results.

Samols & Ryder (230) have presented a method for simultaneous determination of exogenous and endogenous insulin by Berson & Yalow's technique. One test system uses human anti insulin prepared

from serum from insulin treated patients. This anti insulin has only a very slight affinity for human insulin, and its use for measuring the immunologically active insulin in human serum means that only exogenous insulin is measured, not human insulin. Another test system uses guinea pig anti insulin, which has a considerable affinity for both human and non human insulin, whereby both exogenous and endogenous insulin are measured. The amount of endogenous insulin is thus calculated as the difference between the measurements by the two test systems.

The immunological methods described are based on the assumption that the serum insulin which can react with insulin antibody has immunological characteristics identical with those of the insulin extracted from pancreas, which is used for production of the standard insulin solutions. Recent investigations have raised doubts as to the validity of this assumption. Moloney (172) has thus immunized mice against ox insulin. These mice did not develop diabetes, but after injection with mouse pancreas insulin the mice were found to be resistant towards this insulin. Injection of anti insulin produced by injection of ox insulin into guinea pigs elicited a diabetic condition in the mice. This investigation suggests that serum insulin in mice differs immunologically from purified pancreas insulin. It might well be that the immunological difference is due to a change in the insulin molecule, as a result of the extraction processes undergone by the pancreas insulin. This

assumption gains support from investigations which have shown that cows may form insulin antibody against ox insulin, and that this does not cause diabetes in the animals (213). In view of these findings, it must for the present be considered an open question whether the biologically active serum insulin is immunologically identical with the animal pancreas insulin which is used in the test system.

Sensitivity

Insulin amounts greater than $2.5 \mu\text{U/ml}$ can be determined by Arquilla & Stavitsky's method (13). Berson & Yalow (31) report that they can measure $0.1 \mu\text{U/ml}$ by their method. Insulin amounts greater than $20 \mu\text{U/ml}$ can be demonstrated by Grodsky & Forsham's technique (103), whereas insulin concentrations greater than $1 \mu\text{U/ml}$ can be measured by Hales' method and by Morgan & Lazarow's method (175, 313).

Precision

The accuracy of the immunological methods is very great, and here these methods are far superior to the biological methods available. Morgan & Lazarow (175), in repeated determinations on the same serum, found a standard deviation of 1%. Hales & Randle (115) in two investigations by the immunological method, found lambda values of 0.02 and 0.06.

Specificity

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CHAPTER 2

VARIOUS FORMS OF INSULIN IN SERUM

Shortly after investigators had begun using the insulin assays on blood as described in the last chapter, the various methods were found to give rather divergent results. In some of the methods, different physical treatments of plasma apparently caused a considerable rise in its insulin like activity. This raised the question whether plasma insulin might not occur in different forms, and whether there might not be a conversion from one form to another, depending on circumstances both *in vitro* and *in vivo*. It was conceivable that the insulin was transported in plasma partly as free molecules of insulin, partly in another form perhaps bound in some way to certain plasma protein molecules.

To elucidate these circumstances, several investigators examined the insulin activity in different protein fractions separated by precipitation, electrophoresis or resin treatment. The results of these investigations are difficult to evaluate, and they are often incompatible because of methodological differences, but a number of investigations nevertheless seem to indicate that insulin is transported in plasma in several different forms.

IL 1 in serum protein fractions prepared by cold ethanol fractionation and by resin extraction

When serum proteins were separated by Lever's method (148) and the ILA examined by the rat diaphragm technique, several investigators found ILA in fraction I+III (α_2 -globulin, β -globulin and fibrinogen) (89, 283), but only Gardiner & co-workers found ILA in fraction II (γ -globulins). Fractionation by Lever's method, followed by electrophoresis on treated cellulose, then testing by the rat diaphragm method, showed ILA in albumin α_1 -globulin isolated from fractions IV, V, and VI (283). Gardiner & co-workers found no ILA in these protein fractions, but they did not perform an electrophoretic separation. As Vargas & co-workers (283) found an insulin antagonist localized to α_2 -globulin in fractions IV, V, VI, it is reasonable to believe that the effect of any insulin which may be present in these fractions is inhibited by the antagonist, unless it is removed by additional fractionation.

The investigations cited do not give

not be demonstrated in serum from pancreatectomized dogs (74) Immunologically-determined insulin disappears after treatment of serum with cysteine If insulin is added to serum, the immunologically-determined insulin rises by an amount corresponding to the insulin added (31) In normal subjects, ingestion of glucose is followed by a rise in the amount of immunologically-determined insulin in serum (175, 308) With Morgan & Lazarow's method, serum from alloxan-diabetic rats showed lower values for immunologically determined insulin than serum from normal animals (175)

Grodsky & co-workers, using their own technique, found a rise in the values for immunologically active insulin in the pancreatic vein, on infusing a solution of glucose into isolated rat pancreas (104)

It is of considerable interest that Yalow & Berson (310) showed that an insulin derivative, desoctapeptide insulin, which has no biological effect, can be demonstrated by the immunological method, whereas neither the insulin's A chain nor B chain reacts with insulin antibodies Grodsky & co-workers (102) have made similar studies on insulin conversion products They found that insulin treated with carboxypeptidase or dinitro-benzolsulphonate, or insulin which has undergone "mild esterification", can be bound to insulin antibodies This holds also for acetylated insulin and diazotized insulin

By means of their immunological methods, Yalow & Berson (308) and Hales & Randle (113) found that the content of immunologically-determined insulin in serum decreases proportionally with the dilution Examining undiluted rat serum, Morgan & Lazarow (175) obtained very high values for immunologically-determined insulin, lower values were obtained when the serum was diluted This was found to depend on the presence in rat serum of a factor different from insulin, and influencing the test system The effect of this factor seems to disappear on dilution A corresponding phenomenon appears to be lacking in human serum (242)

CONCLUSION

From the results of the above investigations, it seems a reasonable conclusion that there is every probability that other than insulin, normal serum contains no factors which can be registered as insulin-like activity by the rat diaphragm and the rat epididymal fat methods There is likewise every probability that the immunological methods based on Berson & Yalow's principle are specific for insulin in normal serum

No sufficient experimental basis exists for drawing the same conclusion as to the *in vivo* methods or Arquilla & Stavitsky's immunological method

effect on rat diaphragm, it had an insulin like activity when examined by the rat epididymal fat method. In a subsequent investigation, the same investigators found that fatty tissue, in contrast to muscle tissue, contains a factor which can activate the hidden insulin-like activity in resin eluates (10). Shaw & Shucy (236) confirmed that this fatty tissue extract can cause a considerable rise in the insulin like activity of serum.

Investigations by Anthoniades & co-workers seem to indicate that serum contains different types of ILA, one type being active towards rat diaphragm in untreated serum, and another type which has no effect on this tissue, both types can be measured by the rat epididymal fat method. Anthoniades put forward the hypothesis that ILA which is adsorbed to the resin, is present in a form bound to a basic protein, and he succeeded in eluting a protein substance from the resin migrating cathodically at pH 7.4 (6). Subsequent electrophoretic investigations (7) however seem to indicate that this basic protein can hardly have significance for the adsorption to the resin.

ILA in serum protein fractions prepared by electrophoresis

A few studies throw some light on the occurrence of ILA in electrophoretically separated serum protein fractions. Randle & Taylor (209-263) performed electrophoresis on columns of treated cellulose and they determined the ILA by the rat diaphragm method. Using this

technique, these investigators found ILA in both the albumin- α_1 globulin fraction and in the β - γ globulins. Lyngsoe (157), by electrophoresis in a polyvinyl chloride block and determination of ILA by the rat epididymal fat method, found ILA in most of the protein fractions examined, though the insulin like activity was distributed into two maxima, corresponding to the serum protein fractions in which Randle & Taylor found ILA. Gjedde (58), with a similar technique, found the same distribution of ILA. Bolinger & co-workers (33), using the rat diaphragm method, found ILA in the β -globulins, but not in albumin- α_1 globulin. Other investigators have separated the serum proteins by a method using paper as carrier, and which allows quite large amounts of serum to be separated. Insulin like activity was found in the β -globulins, using both an *in vivo* method with intact mice, and the rat epididymal fat method (22, 27). The most important discrepancy between these studies thus concerns the insulin like activity in albumin- α_1 globulin. In all those investigations in which ILA was demonstrated in these fractions, the serum proteins were dialyzed at room temperature for more than 24 hours (warm dialysis) after the electrophoresis, a procedure which entails a significant rise in both SILA (97) and ILA in the albumin- α_1 globulin and the β - γ globulin fractions (156). This technique seems not to have been used in other investigations on ILA in electrophoretically separated serum protein fractions, it appears reasonable, there-

sufficient details about ILA in the isolated protein fractions, to permit comparisons with the SILA values which can be demonstrated in unfractionated serum by the same methods. It is of considerable interest, however, that Gardner & co-workers (89) could demonstrate a pronounced rise in the insulin-like activity localized to γ globulin, after *in vitro* oxygenation of venous blood, but not after oxygenation of venous plasma. These oxygenation tests were inspired by Young's demonstration (312) that SILA (the rat diaphragm method) was higher in plasma prepared from venous blood oxygenated *in vitro*, than in plasma prepared from unoxygenated venous blood. This finding was later confirmed in studies by the rat epididymal fat method (225). These experiments seem to show that γ globulin from non-oxygenated venous blood contains ILA in a "hidden" form, *i.e.* without biological effect *in vitro*, and that this ILA can be activated by oxygenation of whole blood.

In determinations of insulin-like activity in serum proteins using alloxan-diabetic, hypophysectomized rats, Bergelmann, Anthoniades, Goetz, Renold, Oncley & Thorn (26) found ILA in fractions II+III (β - and γ -globulins). In this investigation, the serum proteins were separated by cold ethanol fractionation using methods 6 and II (60). By additional fractionation, ILA was found in the β -lipoprotein containing fraction III-0. In the same study, ILA was also found in the fraction IV-4 (albumin- α_1 -globulin, α_2 globulin and

β_1 -globulin). It was found that while there was ILA in fraction II+III isolated from citrated blood, ILA could not be demonstrated in the corresponding protein fractions isolated from blood in which the coagulation had been inhibited by treatment with a cation exchange resin (Dowex 50 or IRC 50). This peculiar result was later taken up for further investigation by Anthoniades & co-workers in numerous studies. Anthoniades (5) showed that insulin like activity could be eluted from the resin, and electrophoretic separation showed that this ILA was localized to β - γ globulins (7). Gundersen & Anthoniades (107) found that the resin eluates had no insulin-like activity with rat diaphragm, unless they had previously been exposed to a pH considerably higher than physiological. It was found in a subsequent study that in spite of SILA having the same value in untreated and in resin treated serum, ILA could be eluted from the resin (8). In a subsequent study, other investigators have confirmed that considerable amounts of ILA can be eluted from the resin, much greater amounts than the same investigators find in untreated serum (the rat diaphragm method). They have also shown that this ILA is inhibited by anti insulin (236). These findings seem to indicate that the ILA which can be eluted from the resin, must be present in untreated serum in a 'hidden' condition in which it has no effect on rat diaphragm. Gundersen & Anthoniades (107) further showed that while a resin eluate which had been eluted at an acid pH had no

effect on rat diaphragm, it had an insulin like activity when examined by the rat epididymal fat method. In a subsequent investigation, the same investigators found that fatty tissue, in contrast to muscle tissue, contains a factor which can activate the hidden insulin like activity in resin eluates (10). Shaw & Shuey (236) confirmed that this fatty tissue extract can cause a considerable rise in the insulin like activity of serum.

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fore, to assume that this is why other investigators have not been able to demonstrate ILA in albumin- α_1 -globulin

The observation that the insulin like activity in both the albumin- α_1 -globulin fraction and the β - γ -globulin fraction will rise following "warm dialysis" is of considerable interest. As the ILA is considerably higher in the "warm dialyzed" serum proteins than in untreated serum (157), it may be concluded that the ILA in untreated serum is present in a preponderantly inactive state, which "warm dialysis" changes to an active state. On investigating "warm dialyzed" serum protein fractions by the rat epididymal fat method, it was found that the insulin-like activity, just as in the case of SILA, is partially inhibited by anti insulin. A proportion of the very large amount of ILA determined under these conditions, therefore, is undoubtedly insulin, in the sense that it can combine with insulin antibody in an immunological reaction. As a certain proportionality was found between ILA which was inhibited by anti-insulin and ILA which was not inhibited, it seems reasonable to assume that both forms of ILA are insulin (156). It must be concluded, therefore, that insulin corresponding to both isolated serum protein fractions is present in two forms, one in which it is active immunologically as well as biologically, and one in which it is only active biologically. This is presumably the reason why SILA, in the investigations by the rat epididymal fat method, is only partially inhibited by anti insulin.

Studies by Samaan & co-workers

(229) are of considerable interest in this connection. These investigators measured SILA in serum with and without the addition of anti-insulin. Using this method, they examined the migration of SILA through a membrane which was impermeable to plasma proteins of high molecular weight, but which was permeable to purified insulin in solution. They found that SILA which was inhibited by anti-insulin ("suppressible SILA"—"typical insulin") could penetrate through the membrane, which was impermeable to that part of SILA which was not inhibited by anti insulin ("non-suppressible SILA"—"atypical insulin"). This finding seems to indicate that serum insulin which is present in an immunologically active form, is not attached to high molecular proteins, and that the biologically active (with rat epididymal fat) but immunologically inactive part of the serum insulin is present in a higher molecular state than the dissolved insulin. Therefore, since ILA which is not inhibited by anti insulin can be demonstrated in both albumin- α_1 -globulin and in β - γ -globulin it seems reasonable to conclude that both these protein fractions contain insulin in a high molecular state, possibly bound in some way to the plasma proteins.

DISCUSSION

It appears from the above that in a few studies, it has been demonstrated that serum contains several forms of insulin like activity. Antoniadou's investigations with the rat diaphragm method see

page 36) have thus shown that in untreated serum, besides the SIIA which is immediately demonstrable, there must be a 'hidden' IIA, i.e. an IIA without any effect on the test system used.

Using the rat epididymal fat method, investigators have been able to demonstrate two types of SIIA, one which is inhibited and one which is not inhibited by anti insulin (see chapter 1). Gjerdde, with the same method, showed that SIIA in serum increases after warm dialysis, indicating that in addition to the SIIA which can be immediately demonstrated by the rat epididymal fat method, untreated serum contains hidden IIA. Lyngsoe's investigations show that hidden IIA is present corresponding both to the albumin- α , globulin and to the β - γ globulin fraction.

These findings are compatible with the following hypothesis. Serum contains two forms of insulin. In the one form the insulin is immunologically active in the other form it is immunologically inactive. Immunologically inactive insulin is present in two fractions (A' and B) localized by means of electrophoresis to albumin- α , globulin and β - γ globulin, respectively.

In the rat epididymal fat method, both suppressible and non suppressible SIIA can be demonstrated in untreated serum. This seems to indicate that the method can determine both immunologically active and immunologically inactive insulin. SIIA which is determined by the rat diaphragm method is totally inhibited by anti insulin, this seems to indicate that in untreated serum the latter meth-

od solely determines immunologically active insulin. It has not been possible to demonstrate that serum contains any insulin which is immunologically active but biologically inactive, it is therefore very likely that the IIA which Anthorn and his co-workers found was adsorbed to the resin, and which in untreated serum is without any effect on rat diaphragm, is due to immunologically inactive insulin. This assumption is in accordance with the fact that IIA which is bound to resin, and which cannot be demonstrated by the rat diaphragm method after elution at acid pH, can be determined by the rat epididymal fat method.

As the IIA (the rat epididymal fat method) in serum and in serum protein fractions increases after warm dialysis, serum must contain hidden IIA, as the increase in IIA after warm dialysis can be demonstrated in both albumin- α , globulin and in β - γ globulin, this suggests that insulin is present in an immunologically inactive form in both these fractions. The assumption that immunologically inactive insulin is found in γ globulin gains further support from Gardiner's investigations, which demonstrate that IIA is higher in γ globulin prepared from oxygenated venous blood, than in γ globulin prepared from non-oxygenated venous blood (see page 36). It may be concluded, therefore, that hidden IIA, localized to the γ globulin, must be present in non-oxygenated venous blood.

A possible explanation for the presence of hidden IIA in serum, as demonstrated by resin treatment and by

"warm dialysis", might be that the insulin in serum is inhibited by insulin antagonists (see chapter 3). A considerable part of the hidden ILA which can be demonstrated by the rat epididymal fat method after "warm dialysis", however, is not inhibited by anti-insulin, and this cannot be explained as a consequence of insulin antagonism. Furthermore, as insulin antagonists with an effect on rat epididymal fat are not known in normal serum, it is hardly probable that the presence of hidden ILA (the rat epididymal fat method) is due to insulin antagonism. On the other hand, it cannot be excluded that the hidden ILA (the rat diaphragm method), demonstrable by resin treatment of serum, may be due to insulin antagonism, although it is hardly probable.

From the preceding discussion, it appears that a measure of the immunologically inactive insulin in serum may be obtained by three different methods. This raises the question whether these three methods determine the same or different fractions of the immunologically inactive insulin in serum. Anthoniades (7) reports that ILA which is adsorbed to the resin, migrates electrophoretically so as to correspond to β_1 -globulin. Lyngsøe (158), on treating serum with resin, did not find any definite fall in ILA either in warm dialyzed albumin- α_1 globulin or in warm dialyzed β_1 -globulin. It has not been established, however, that it is possible to determine all immunologically active insulin in serum protein fractions which have been subjected to warm dialysis. Thus

the investigation in question does not exclude the possibility that part of the immunologically inactive insulin in the β_1 -globulin fraction is removed following resin treatment of serum. Samaan & co-workers (229), using the rat epididymal fat method, examined suppressible and non-suppressible SILA in serum before and after treatment with resin, and they were unable to demonstrate any fall in the amount of non-suppressible SILA. Neither does this investigation exclude the possibility that part of the immunologically inactive insulin disappears from serum on resin treatment. It is thus possible that the insulin-like activity which can be eluted from the resin, is a measure of the immunologically inactive insulin in the β_1 -globulin fraction.

No studies are available to elucidate whether non-suppressible SILA determined by the rat epididymal fat method, is a measure of the immunologically inactive insulin in albumin- α_1 globulin or in β_1 -globulin, or whether it is a measure of both insulin fractions, although the latter possibility seems to be more likely.

As mentioned, the investigations discussed seem to indicate that immunologically inactive insulin is found corresponding to both albumin- α_1 globulin and to β_1 -globulin. There are scanty references in the literature to the electrophoretic localization of immunologically active insulin. Anthoniades (7) in a very short report mentions that free insulin (i.e. SILA which is not adsorbed to resin) migrates corresponding to al-

bumin and globulin Berson & Yalow (31) examined serum by their immunological method and showed that immunologically determined insulin and added I^{131} -labelled insulin are found on ultracentrifugation to be distributed in the same manner. This finding perhaps supports the assumption that the immunologically active insulin in serum, and I^{131} labelled insulin, also migrate electrophoretically in an identical way. As I^{131} labelled insulin added to serum migrates corresponding to albumin- α_2 -globulin (4, 29, 158-210) it is possible that the immunologically active insulin in serum is found in the same protein fraction. It is probable therefore, that albumin- α_2 -globulin contains both immunologically active and immunologically inactive insulin.

Our knowledge of molecular size and possible protein binding of immunologically active and immunologically inactive insulin is very limited. The studies of Samaan & co-workers on the diffusion characteristics of suppressible and non

suppressible SIIA (see page 38) suggest that insulin has a smaller molecular size in the immunologically active form than in the immunologically inactive form.

Anthoniades (7) reported that investigations with Sephadex seem to indicate that IIA adsorbed to resin has a molecular weight of more than 40-60,000, whereas IIA not adsorbed to resin has a lower molecular weight. These investigations might suggest that insulin in the immunologically inactive form is bound to high molecular proteins, in contrast to immunologically active insulin. Other studies, however, tell against so simple an interpretation of the state of serum insulin. Berson & Yalow (31), on ultracentrifugation, found only 70% of the immunologically-determined insulin in the supernatant above the albumin layer. Their investigation thus seems to show that a smaller part of the immunologically active insulin in serum is present in a high molecular form.

warm dialysis, might be that the insulin in serum is inhibited by insulin antagonists (see chapter 3). A considerable part of the hidden ILA which can be demonstrated by the rat epididymal fat method after 'warm dialysis', however, is not inhibited by anti insulin, and this cannot be explained as a consequence of insulin antagonism. Furthermore, as insulin antagonists with an effect on rat epididymal fat are not known in normal serum, it is hardly probable that the presence of hidden ILA (the rat epididymal fat method) is due to insulin antagonism. On the other hand, it cannot be excluded that the hidden ILA (the rat diaphragm method), demonstrable by resin treatment of serum, may be due to insulin antagonism, although it is hardly probable.

From the preceding discussion it appears that a measure of the immunologically inactive insulin in serum may be obtained by three different methods. This raises the question whether these three methods determine the same or different fractions of the immunologically inactive insulin in serum. Anthoniades (7) reports that ILA which is adsorbed to the resin, migrates electrophoretically so as to correspond to β - γ globulin. Lyngøe (158), on treating serum with resin, did not find any definite fall in ILA either in warm dialyzed albumin- α_1 globulin or in warm dialyzed β - γ globulin. It has not been established, however, that it is possible to determine all the immunologically active insulin in serum protein fractions which have been subjected to warm dialysis. Thus,

the investigation in question does not exclude the possibility that part of the immunologically inactive insulin in the β - γ globulin fraction is removed following resin treatment of serum. Samraan & co-workers (229), using the rat epididymal fat method, examined suppressible and non-suppressible SILA in serum before and after treatment with resin, and they were unable to demonstrate any fall in the amount of non-suppressible SILA. Neither does this investigation exclude the possibility that part of the immunologically inactive insulin disappears from serum on resin treatment. It is thus possible that the insulin like activity which can be eluted from the resin, is a measure of the immunologically inactive insulin in the β - γ globulin fraction.

No studies are available to elucidate whether non suppressible SILA determined by the rat epididymal fat method is a measure of the immunologically inactive insulin in albumin- α_1 globulin or in β - γ globulin or whether it is a measure of both insulin fractions, although the latter possibility seems to be more likely.

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localized to the albumin fraction. The antagonist could be demonstrated in albumin from both diabetic patients and normal subjects, though the investigations suggested that it was found in the highest concentration in diabetics (276, 280). In subsequent studies, Vallance Owen & co-workers also found increased concentration of antagonist in albumin from a majority of patients with recently cured myocardial infarction, in pre-diabetics (patients with a family history of diabetes and abnormal oral glucose tolerance after ingestion of cortisone) (281, 282), and in some relatives of patients with diabetes mellitus (282).

It is of considerable interest that while the antagonist described by Vallance Owen has an insulin inhibiting effect in studies with the rat diaphragm method, concurrent investigations show that it does not influence the insulin action on the rat epididymal fat oxidation of glucose C^{14} to $C^{14}O$ (150, 155).

Vallance Owen & Lilley (280) did not find insulin antagonism in albumin from non-substituted adrenalectomized patients whereas albumin from substituted patients was found to be antagonistic. Vallance Owen & co-workers (277) found no insulin antagonism in albumin from hypophysectomized patients but Lowy and co-workers in corresponding studies arrived at the opposite result (150). If the patients examined by Lowy had a milder degree of pituitary insufficiency than those examined by Vallance Owen, this would no doubt account for the findings. The publications cited however do not con-

tain the information necessary to support such an assumption.

While Lowy & co-workers (150) succeeded in finding insulin antagonism in serum albumin, Keen (132), in a recently published study, has been unable to confirm this finding. On the contrary, he found that the extracted albumin contained insulin. The reason for the absence of agreement between these studies has not been elucidated. It must be emphasized, however, that after the extraction of albumin, Vallance Owen performs a vacuum extraction which entails considerable foam formation, and which presumably alters a proportion of the protein molecules, whereas Keen omitted this procedure. This difference of technique may be significant as the most recent studies by Vallance Owen (77) suggest that the B chain of insulin has an insulin antagonistic effect on rat diaphragm, and that the

Vallance Owen antagonist has a number of physico-chemical characteristics in common with the B chain. Thus, though these findings are compatible with the assumption that the Vallance Owen antagonist is a break down product of insulin, formed during the extraction procedure, this hypothesis does not explain the insulin antagonism in untreated serum from non-ketotic diabetics and the dependence of the insulin antagonist on the adrenal cortex function. Further work on these problems will therefore be of the very greatest interest.

Field & co-workers, using Stadie's modification of the rat diaphragm meth-

CHAPTER 3

SERUM INSULIN ANTAGONISTS

In determinations of SILA before and after the addition of small quantities of insulin to serum, the added insulin is in most cases recovered quantitatively. In corresponding studies on serum from diabetic patients, it was found in some cases that no rise in SILA could be demonstrated after the addition of insulin. It was concluded that the effect of the added amount of insulin was inhibited by the serum. The inhibiting factors were designated insulin antagonists. In the present study, this designation is only used for such serum factors as inhibit the action of insulin on isolated tissue *in vitro*.

Studies published during recent years have demonstrated that insulin antagonists are present in the serum from both normal subjects and diabetic patients. Up till now, 5 insulin antagonists have been described in man, but a closer comparison of these possibly different, possibly identical antagonists, is hampered by the fact that only in a few exceptional cases have investigators attempted to compare their own results with the results of others. It is of considerable interest that insulin antagonism has also been found in diabetic experimental animals, e.g. the rat and the cat.

Later on in the present chapter, a short summary will be given of the significance of insulin antibody as an insulin antagonist in man, although this *anti-insulin* is a *iatrogenic phenomenon*, without significance for our understanding of the metabolic abnormalities in diabetes mellitus.

Human serum insulin antagonists

Bornstein (36), examining a number of poorly regulated diabetics, found that the serum from these patients lacked SILA, and that when it was injected into ADHA rats, the sensitivity of these animals towards insulin was decreased for up to 6 weeks after the injection.

Vallance-Owen has published numerous studies on serum insulin antagonism. In all these studies, the author and his co-workers used the rat diaphragm method. Using this technique he demonstrated that undiluted serum from poorly regulated, non-ketotic diabetic patients inhibited the action of added insulin, and that the inhibiting effect disappeared after dilution (270, 277). Using tri-chloroacetic acid fractionation (68) and cold ethanol fractionation (60), it was found that the antagonistic effect was

localized to the albumin fraction. The antagonist could be demonstrated in albumin from both diabetic patients and normal subjects, though the investigations suggested that it was found in the highest concentration in diabetics (276, 280). In subsequent studies, Vallance Owen & co-workers also found increased concentration of antagonist in albumin from a majority of patients with recently cured myocardial infarction, in pre diabetics (patients with a family history of diabetes and abnormal oral glucose tolerance after ingestion of cortisone) (281, 282), and in some relatives of patients with diabetes mellitus (282).

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Field & co-workers, using Stadie's modification of the rat diaphragm meth-

od (see page 17), found insulin antagonism in serum from patients with diabetic acidosis (81) Field described a number of different characteristics of this antagonist In starch block electrophoresis, it migrated corresponding to the α_1 -globulin fraction, it was not dialysable, and it was destroyed by boiling or by treatment with chymotrypsin, but not by treatment with trypsin (82) The antagonist was not destroyed by repeated freezing and thawing of serum and was not localized to the lipoprotein fraction The antagonist had no insulinase action, and as it did not inhibit the binding of I^{131} -labelled insulin to rat diaphragm, the antagonistic effect is hardly due to a binding between insulin and antagonist (81) Field & Rigby (83), using a more sensitive technique than in the first studies, found insulin antagonism in serum from a few poorly controlled, non-acidotic, juvenile diabetics with ketonuria In the same study, insulin antagonism was demonstrated in a few patients with acute infectious diseases, as well as in three out of four patients with acromegaly and diabetes

Examining serum from acidotic diabetics, later investigators have been able to demonstrate an insulin antagonist which may be identical with the factor which Field characterized in detail Thus, unfractionated serum from such patients will inhibit the insulin effect on the glucose uptake of rat diaphragm (108, 292), whereas no insulin antagonism can be demonstrated in electrophoretically separated serum protein fractions from acidotic diabetics (292)

Studies by the rat epididymal fat method on undiluted serum from acidotic diabetics showed a pronounced insulin antagonistic effect, which disappeared, however, after five fold dilution of the serum (155) In accordance with this finding, Samaan et al (229) were unable to demonstrate insulin antagonism in diluted serum from ketotic diabetics by means of the rat epididymal fat method

Serum samples from patients with diabetic coma and from a few normal subjects were fractionated by Lever's method (148) A factor was found in the α_2 -globulin which inhibited the glucose uptake of rat diaphragm (261, 264, 283) The factor is not present in serum from hypophysectomized patients unless the patients are given treatment with growth hormone (264) This factor is presumably insulin antagonistic, like the factors described above, but no studies are available which demonstrate a direct insulin-inhibiting effect

Baird & Bornstein (16) examined serum from patients with diabetic coma by means of an alcohol toluene extraction They found that while the extract contained SIAL, the remainder contained a factor which inhibited the glucose uptake of rat diaphragm In a subsequent study, Bornstein & Hyde (43) found that serum from non-acidotic juvenile diabetics with long term diabetic manifestations contained this inhibiting factor whereas serum from juvenile diabetics without long term diabetic manifestations, but with the same duration of diabetes, lacked the factor It is regret

table that these interesting investigations, which seem to indicate that serum contains an antagonist significant for the development of long term diabetic manifestations, have not been taken up by other investigators.

Recently published studies suggest that an increased concentration of free fatty acids (NEFA) will inhibit the insulin effect on rat diaphragm *in vitro* (211). This finding makes it reasonable to assume that in serum from certain poorly regulated diabetics without acidosis the insulin antagonism which can be demonstrated by means of the rat diaphragm method may be due to an increased level of NEFA. At present there are no studies available on comparisons between NEFA and serum insulin antagonism in diabetics, so that the importance of NEFA for insulin antagonism cannot be considered as proved.

As previously mentioned (see page 22), some studies are available showing that epinephrine in physiological concentrations will inhibit the insulin effect on the glucose uptake in rat diaphragm. Epinephrine must therefore be characterized as an insulin antagonist (106). Epinephrine has no insulin antagonistic effect on the glucose metabolism of rat epididymal fat (see page 30).

It should be mentioned finally that using both *in vivo* methods the rat diaphragm method and the rat epididymal fat method, it is possible to demonstrate insulin antagonism in serum from diabetics who have developed insulin resistance after having been treated with insulin for some time (61, 79, 80, 84,

131, 134, 149, 155, 165, 176, 232, 311). The insulin antagonist has the nature of an antibody, and is localized to the γ globulin or to the inter β - γ globulin fractions (46, 61, 84, 131, 150, 232). Berson and Yalow have by an electrophoretic technique demonstrated insulin antibody in β - γ globulin in all diabetics as early as three months after the start of insulin treatment (29), although the quantity of insulin antibody in serum from insulin resistant diabetics is considerably higher than in serum from insulin sensitive diabetics (310). Even though serum from patients who have had insulin for a long time, and who are non insulin resistant, is without antagonistic effect on minor quantities of insulin (155), it seems reasonable to assume that the insulin antibody demonstrated by Berson & Yalow causes the insulin antagonism in serum from insulin resistant patients.

Serum insulin antagonism in experimental animals

Examining alloxan diabetic rats, Tuerkischer & Wertheimer (265) and Stadie & co-workers (247) found that serum from these animals inhibited the insulin effect on the glycogen synthesis in normal rat diaphragm. Bornstein & Park (41) found that serum from alloxan-diabetic rats inhibited the glucose uptake in normal rat diaphragm. These and later investigations showed that the inhibiting factor was dependent on both pituitary and adrenal glands, as serum from hypophysectomized or adrenalectomized rats did not inhibit the glucose

uptake of rat diaphragm, unless the animals received substitution therapy with growth hormone and cortisone, which were without effect *in vitro*, however (41, 300) In subsequent studies, investigators succeeded in localizing the inhibiting factor to β_1 -lipoprotein from both alloxan diabetic rats and normal rats (37, 138) These last-mentioned investigators found that the inhibiting factor reduced the effect of small amounts of insulin on the glucose uptake of rat diaphragm On the other hand, the antagonist is without any effect on rat epididymal fat (133)

Several investigators have shown that the β_1 -lipoprotein-antagonist loses its effect on repeated freezing and thawing of serum (37, 120) This is presumably why an examination of fresh serum from alloxan-diabetic rats shows a decreased SILA, whereas normal values of SILA are found after repeated freezing and thawing of serum (201)

Vallance-Owen & Lukens (275) demonstrated insulin antagonism in serum from pancreatectomized cats This insulin antagonism has much in common with the β_1 -lipoprotein antagonist in rats and one thing it cannot be demonstrated following hypophysectomy or adrenalectomy The antagonist effect, however, was not recovered when hypophysectomized pancreatectomized cats had been treated with growth hormone or after adrenalectomized-pancreatectomized cats had been treated with cortisone or hydrocortisone, and the antagonistic effect did not disappear after repeated thawing and freezing of serum

DISCUSSION

It is not possible to carry out a systematic comparison between the serum insulin antagonists which have been described in man, both normal subjects as well as diabetic patients, and in experimental animals The studies made are too incomplete for this purpose

One of the best characterized antagonists is the one described by Field in patients in diabetic acidosis It has been discussed whether this antagonist, which is localized to α_1 globulin, is identical with the antagonist described by Vallance-Owen in the albumin fraction An insulin antagonist which is active with rat epididymal fat, however, is present in patients in diabetic acidosis, and as this epididymal fat is not affected by the antagonist described by Vallance-Owen (155), it seems reasonable to assume that Field's and Vallance Owen's antagonists are different Field's antagonist is not destroyed after repeated freezing and thawing of serum, and it is not localized to the lipoprotein fraction, this antagonist is therefore undoubtedly different in character from that of the lipoprotein antagonist which has been demonstrated in alloxan-diabetic rats It may not be possible to demonstrate an antagonist in experimental animals analogous to the antagonist in man, as described by Field, but investigations with a view to further characterizing the insulin antagonism which can be demonstrated in pancreatectomized cats will also be of considerable interest in this connection

Bornstein (37) examined lipoproteins in normal subjects for the purpose of finding insulin antagonism similar to the type which has been demonstrated in rats. He succeeded in demonstrating such an antagonist in one psychotic patient in insulin hypoglycaemia, but not in other normal subjects. This interesting finding seems not to have been followed up by other investigators.

It is of the greatest interest that serum insulin antagonists which are dependent on hormones have been demonstrated in both man and experimental animals. For example, the β -lipoprotein antagonist in the rat, the antagonist in the pancreatectomized cat and perhaps even Vallance Owen's antagonist, all depend on an intact pituitary as well as on intact adrenal glands, while Taylor's α_2 globulin factor in man seems to depend on the pituitary. Further investigations are therefore necessary before the possibility of a relationship between these antagonists can be rejected.

The demonstration of insulin antagonism in serum from diabetic patients, and in particular Vallance Owen's in

vestigations on the albumin antagonist, have resulted in the phenomenon "insulin antagonism" being introduced into the discussion on the pathogenetic factors in diabetes mellitus. The hypothesis has been put forward that an increased production of insulin antagonist in the pre diabetic and the diabetic condition will lead to a surplus production of insulin, which will gradually entail an exhaustion of the pancreatic tissue, resulting in secondary insulin deficiency. In spite of the fact that there are a number of studies which favour such a hypothesis, investigations on insulin antagonism in animals show that insulin antagonism in serum may occur in situations where a primary insulin deficiency must be assumed, as for example following a pancreatectomy or alloxan treatment. If analogies are drawn to patients with diabetes mellitus, then based on the finding of animal experiments it may be supposed that the cause of the serum insulin antagonism in certain diabetic patients is a reduced production of insulin, although the reverse mechanism is of course also conceivable.

CHAPTER 4

INSULIN CONTENT OF BLOOD FROM NORMAL SUBJECTS AND EXPERIMENTAL ANIMALS

The determination of the insulin content of the blood involves a number of problems. For one thing, some of the serum insulin is present in a state of biological and immunological inactivity (see chapter 2), and for another, the serum may contain insulin antagonists which will inhibit the biological effect of the insulin, and thus influence the determination of serum insulin by biological methods (see chapter 3). As a result of these two circumstances, untreated serum examined by biological methods will give a combined measure of the effect of the biologically active insulin and any possible insulin antagonists in the serum; this activity has been designated *SILA*. An examination of untreated serum by immunological methods determines only the immunologically active insulin in the serum. But this insulin fraction represents only a very small part of the serum insulin, and should therefore not be considered a measure of the insulin content of the serum. Determination of the total insulin content of serum should be made under circumstances in which all the serum insulin is present in an active

form, immunologically or biologically. If a biological method is used, it should be a condition that the effect of serum insulin antagonists is eliminated. The aim of insulin extraction methods is to achieve such circumstances, and using these methods, some investigators have obtained very high values for immunologically determined insulin and biological insulin-like activity in the serum extracts. In diluted serum which has undergone prolonged dialysis at room temperature, *ILA* values have been obtained of an order of magnitude equal to the highest values measured after insulin extraction. At present, the values which have been obtained in serum extracts and after "warm dialysis" of serum must be considered as the most exact measure of the total insulin content of serum, but it is possible that these methods are unable to activate all the insulin in serum.

A large proportion of the insulin in albumin- α_2 -globulin (the *I* fraction) and in β - γ globulin (the *II* fraction) is found in an inactive condition, presumably bound to serum proteins (see chapter 2). Determining the insulin content

of these fractions, therefore, the investigator will experience the same difficulties as in determining the total insulin content of serum. The only investigations which have been made on insulin in the A and B fractions, have been carried out by warm dialysis and by using the rat epididymal fat method. As previously mentioned, it is not certain that all the insulin in these fractions is determined by this technique, even though the values obtained are very high, since the possibility cannot be excluded that the measurements are influenced by insulin antagonists. The activity which is determined by this technique has therefore been designated insulin like activity.

A number of studies seem to indicate that serum contains a small amount of immunologically active insulin (see chapter 2). Using the immunological methods for examining serum, only this form of insulin will be determined. It is reasonable to assume that the part of SILA which is inhibited after addition of anti insulin to serum, suppressible SILA, is also a measure of the immunologically active insulin.

In the published studies on the insulin content of serum, the composition of the normal material has varied in a number of ways. Some investigators have used serum from healthy subjects, others have taken it from patients with diseases which presumably do not influence the serum insulin. In the present account, persons are considered as normal if they do not suffer from diabetes mellitus or other endocrine diseases. On the

other hand, since Western civilizations have a diabetes mellitus incidence of only 1-2%, and since the incidence of other endocrine diseases scarcely surpasses this figure, material has been accepted as "normal", even though these two patient groups are not explicitly excluded. Consequently, obese non diabetic subjects are included in the normal series, in spite of the fact that SILA values in such patients are different from those found in non-obese normal subjects (see chapter 6). This state of affairs must likewise be accepted, for in by far the majority of publications on SILA in normal subjects, it is not possible to distinguish between obese and non-obese subjects.

A number of studies are available which provide information on the serum insulin in experimental animals. Some of these studies have led to significant information on the secretion of insulin and they are summarized at the end of this chapter.

SERUM INSULIN IN NORMAL SUBJECTS

SILA in peripheral venous blood

The insulin like activity determined in untreated blood, serum or plasma by biological methods is designated SILA, whether the investigations have been made with undiluted or with diluted medium.

Tables 5 and 6 illustrate the SILA investigations so far published on peripheral venous blood from normal subjects. There are a few investigations on SILA

in blood from other parts of the human circulation, these are summarized on page 62. It appears from tables 5 and 6 that most SILA studies have been on serum, but that a few measurements using *in vivo* techniques and the rat diaphragm method have been done on heparinized plasma. Whether investigations on serum and plasma are comparable has not been established. Preliminary results with the rat epididymal fat method seem to indicate that heparin increases the insulin-like activity in serum (168), and heparin appears able to change "complex insulin" (i.e. ILA which is adsorbed to resin) so that it can be measured by the rat diaphragm method (109).

Comparing SILA in undiluted and in diluted serum, allowance must be made for the fact that after correction for the dilution factor, SILA is higher in diluted than in undiluted medium, both by the rat diaphragm method and the rat epididymal fat method. This "dilution effect" was first demonstrated in investigations using the rat diaphragm method (203, 295, 304). Ball & Merrill (19), studying a small experimental series by the rat epididymal fat method (net gas exchange), found a definite dilution effect. Subsequent comprehensive studies confirmed this finding (20, 153, 155, 157), although a few minor experimental series showed no statistically significant dilution effect (152, 240, 252). Steinke & co-workers (254), in a subsequent major study, examined diluted serum from one group of normal subjects and undiluted serum

from another group of normal subjects. In the study cited, the mean figure for SILA was found to be higher in diluted than in undiluted serum, although the difference was not statistically significant. In view of the fact that this study was carried out on two different groups, the result of the investigation does not refute the theory that SILA measured by the rat epididymal fat method is higher in diluted than in undiluted serum.

It is not known why SILA is higher in diluted than in undiluted serum. It might be considered that dilution results in activation of the immunologically inactive insulin, so that it becomes biologically and immunologically effective. This is hardly the case, however, since dilution of serum does not result in a rise in the immunologically-determined insulin (115, 308). The fact that a dilution effect can be demonstrated by the rat diaphragm method was taken to indicate that the effect of one or more insulin antagonists disappears in diluted serum. This assumption gained experimental support when Groen & co-workers (106) showed that the insulin antagonistic effect of epinephrine in physiological concentrations, disappears at a dilution where the insulin effect is not lost. It is possible, therefore, that the dilution effect which can be demonstrated by the rat diaphragm method is due to an elimination of the insulin antagonistic effect of the epinephrine. It may be considered that the dilution effect which can be determined by the rat epididymal fat method, is likewise due

to a 'diluting away' of an insulin antagonist, although in normal serum no insulin antagonists are known which have an effect on this tissue (see chapter 3). It seems more likely, however, that the dilution effect in determinations by the rat epididymal fat method depends on another factor, namely an activation of the immunologically (and biologically) inactive insulin, so that this insulin develops biological activity with respect to fatty tissue. Thus, it has been demonstrated that fatty tissue contains a factor which is able to activate 'complex insulin' (107). As large quantities of insulin are present in serum in an immunologically and biologically inactive condition, it could be conceived that on incubation of fatty tissue with diluted serum, the insulin activating factor would activate part of this insulin.

Studies by the rat epididymal fat method have demonstrated a dependence between SILA levels in undiluted and in diluted serum (153). Rising values of SILA in undiluted serum were associated with rising values in diluted serum. This finding is compatible with the assumption that the dilution effect is due to a diluting away of an insulin antagonist but it is also compatible with the hypothesis of an insulin activation as the cause of the dilution effect.

It appears from table 5 that very few investigations have been made on SILA by *in vivo* methods. These investigations were carried out on undiluted serum, heparinized plasma and whole blood. The mean values for SILA in

these studies vary between 100 and 186 $\mu\text{U/ml}$ in fasting normal subjects. It is shown by Bornstein's method that there is a rise in the SILA level after glucose intake.

Most studies by the rat diaphragm method have been made using Vallance-Owen's modification. With this method, fairly good agreement has been found between SILA values in undiluted plasma, in fasting normal subjects the mean values vary between 60 and 120 $\mu\text{U/ml}$. A single study, but comprising only 5 subjects, shows lower values (233). Wright (304), using Vallance-Owen's technique, found higher values in diluted fasting serum than in undiluted serum, viz a mean value of 200 $\mu\text{U/ml}$.

Groen, Willebrands & co-workers, using other modifications of the rat diaphragm method, found fasting values in diluted serum which were considerably higher than those obtained by Vallance-Owen's technique. Randle (203) examined both diluted and undiluted plasma from normal subjects whose metabolic state was not known. It appears from tables 5 and 6 that Randle's values are significantly higher than those found by Vallance-Owen's method, both for fasting serum and for serum samples taken after ingestion of glucose.

It appears from table 5 that ingestion of glucose causes a rise in the level of SILA in undiluted venous plasma from normal subjects. Anthoniadou & co-workers (9, 11, 12) found a rise in SILA level after intravenous administration of glucose in normal subjects.

TABLE 5
SILA in undiluted blood serum or plasma from normal subjects

Method	Medium	Fasting values		Values after the oral administration of glucose				
		U ml	Mean value	1 hour after glucose		1 1/2 hours after glucose		
				μU ml	Mean value	μU ml	Mean value	
<i>In vivo</i>								
H A D rats ¹⁾	Blood		100					Geilhorn et al. (1941) ²²
ADH rats	Plasma		100(4)		340(4)			Bornstein (1950) ²³
do	do					240-310	340(14)	Bornstein & Larence (1951) ²⁴
Glycogen deposition in rat adipose	Serum	<100-360	166(20)					Rafaelson (1951) ²⁵
<i>Rat diaphragm method</i>								
Vallance Owen's method	Plasma	0-150	67(15)	0-800	445(17)			Vallance Owen et al. (1954) ²⁶
do	do	0-250	94(18)					Wright (1950) ^{27a}
do	do	45-140	85(15)					Wright (1957) ^{27b}
do	do	11-240	59(12)	71-1000	573(12)			Seltzer & Smith (1959) ²⁸
do	do		24(5)					Seltzer (1961) ²⁹
do	do		30(11)					Rizzo et al. (1962) ³⁰
do	do	<100-200	127(12)					Renold et al. (1957) ³¹
do	Serum	10-400	94(9)					Antonades et al. (1961 & 1962) ³²
do	do	0-120	48(15)					Shaw & Shuey (1963) ³³
Randle's method	Plasma	500-6700	2000(5) ³⁴					Randle (1957) ³⁵
<i>Rat epididymal fat method</i>								
Glucose tolerance	Serum	33-940	290(38)					Sieps et al. (1960) ³⁶
C O ₂	do		290(46)					Stenke et al. (1961) ³⁷
	do	20-800						Humbel (1959) ³⁸
	do	42-182	101(10)			0-520	136(23)	Lyngsoe (1961) ³⁹
	do	0-290	71(73)					Lyngsoe (1962) ⁴⁰
	do	25-245	100(18)	32-460	188(11)			Lyngsoe (1964) ⁴¹
	do		482(16)		86(16)			Hear (1962) ⁴²
Net gas exchange	do	75-100	90(5)					Ball & Merrill (1961) ⁴³

The figures in brackets indicate the number of sera investigated

¹⁾ Metabolic state unknown

²⁾ Hypophysectomized adrenomedullarized rats.

TABLE I

Method	Minum	Pa e al no		Values after d e n s u m m a t i o n		U n	M t a n	M t a n	U n	M t a n	U n	M t a n	U n
		u l m	u l m	u l m	u l m								
<i>h a d d i s' a g e n m e t h o d</i>													
Valla e-ethers	Fla III		200 9										Wright (1900) ¹⁰⁴
Ow iod f a m		63-630 87											Groen et al (1902) ¹⁰⁵
Ow x i f a u	Seru	100-3000	1400(14)										W Hebranda et al (1906) ¹⁰⁶
Ow n l i c a	Seru n	300-4600	2300 9)										W Hebranda et al (1908) ¹⁰⁷
Ow x i f a n	Fla a												W Hebranda et al (1908) ¹⁰⁸
Ow x i f a n	Fla a	3300-5700	3900(10)										W Hebranda et al (1908) ¹⁰⁹
<i>J a d e p l d y n a m i c m e t h o d</i>													
Net gas ex l ge	Seru	173-280	218 5)										W Hebranda et al (1908) ¹¹⁰
		0-1400	1119(5)										W Hebranda et al (1908) ¹¹¹
		63-238	750(26)										W Hebranda et al (1908) ¹¹²
			152(15)										W Hebranda et al (1908) ¹¹³
			193(22)										W Hebranda et al (1908) ¹¹⁴
		88 213	153(15)										W Hebranda et al (1908) ¹¹⁵
		< 0-910(22)	193 1)										W Hebranda et al (1908) ¹¹⁶
		107 33	207(9)										W Hebranda et al (1908) ¹¹⁷
		100-800	370(10)										W Hebranda et al (1908) ¹¹⁸
		< 0-910(22)	333(30)										W Hebranda et al (1908) ¹¹⁹
		130-103 15)	450(6)										W Hebranda et al (1908) ¹²⁰
		290 0	139 10)										W Hebranda et al (1908) ¹²¹
		0-430	171(75)										W Hebranda et al (1908) ¹²²
		0-605	187(18)										W Hebranda et al (1908) ¹²³
		0-300	342 2000										W Hebranda et al (1908) ¹²⁴
		60-810	342 2000										W Hebranda et al (1908) ¹²⁵

TABLE 5
SIL 4 in undiluted blood, serum or plasma from normal subjects

Method	Medium	Fasting values		Values after the oral administration of glucose				
		μ U/ml	Mean value	1 hours after glucose		1½-2 hours after glucose		
				μ U/ml	Mean value	μ U/ml	Mean value	
<i>In vivo</i>								
H A D rats ¹⁾	Blood		100					Gellhorn et al (1941) ²²
ADHA rats ²⁾	Plasma		100(4)	340(4)				Bornstein (1950) ²⁴
do	do					240-310	340(14)	Bornstein & Lawrence (1951) ²⁵
Glycogen deposition in rat diaphragm	Serum	<100-365	166(20)					Rafaelson (1961) ¹⁰⁰
<i>Rat diaphragm method</i>								
Vallance Owens modification	Plasma	0-150	67(15)	0-800	445(17)			Vallance Owen et al (1954) ²²¹
do	do	0-250	94(18)					Wright (1950) ²²²
do	do	45-140	85(15)					Wright (1957) ²²³
do	do	11-240	59(12)	71-1000	573(12)			Seltzer & Smith (1959) ²²⁴
do	do		24(5)					Seltzer (1961) ²²⁵
do	do		30(11)					Rizzo et al (1962) ²²⁶
do	do	<100-200	127(12)					Renold et al. (1957) ²²⁷
do	Serum	10-400	94(9)					Anthoniadou et al (1961 & 1962) ²²⁸
do	do	0-120	48(15)					Shaw & Shuey (1963) ²²⁹
Randle's modification	Plasma	500-6700	2000(5) ¹⁾					Randle (1957) ¹⁰⁶
<i>Rat epididymal fat method</i>								
Glucose 1 C ¹⁴	Serum	33-940	290(38)					Sheps et al (1960) ²³⁰
C ¹⁴ O ₂	do		290(46)					Steinke et al (1961) ²³¹
	do	20-800						Humbel (1959) ²³²
	do	42-182	101(10)			0-520	130(23)	Lyngsøe 1961 ²³³
	do	0-290	71(73)					Lyngsøe (1962) ²³⁴
	do	25-245	100(18)	32-460	188(11)			Lyngsøe (1964) ²³⁵
	do		482(16)		786(16)			I hear (1962) ²³⁶
Net gas exchange	do	75-100	90(5)					Ball & Merrill (1961) ²³⁷

The figures in brackets indicate the number of sera investigated

¹⁾ Metabolic state unknown

²⁾ Hypophysectomized adrenalectomized rats

of serum which was similar to the one used by Renold's group, Lyngsøe (152) found normal values of an order of magnitude similar to those found by the group mentioned, whereas Gjedde (98), using serum prepared at 4° C, found SILA values which correspond to the values published in Lyngsøe's studies

It is not known why SILA measured by the rat epididymal fat method is dependent on the method for preparation of serum. It could be that an activation of the immunologically inactive insulin takes place at room temperature, or that an insulin antagonist is destroyed, or that the immunologically inactive insulin is brought to a state in which it is more easily activated by the insulin activating factor of the fatty tissue (see page 37). It is not known whether SILA determined by the rat diaphragm method also depends on the method for preparation of serum, but it is of interest that investigations using Groen's and Willebrands' modifications of this method have all been with serum, whereas the studies using Vallance Owen's modification which gave lower normal values for SILA were made with heparinized plasma.

Most studies using the rat epididymal fat method do not give any details for the preparation of serum. It is therefore impossible to decide whether the variation in normal values found by different workers merely results from the serum used being prepared in different ways. Reviewing the studies published, however, it is of interest to observe that all investigators using Renold's method

without modifications (66, 190, 237, 254) obtain almost similar normal values, whereas other groups who have worked out modifications of the rat epididymal fat method, obtain lower and identical normal values (19, 47, 98, 152, 153, 157, 198, 227).

A single material of normal subjects was examined with respect to variations in age and sex (153). No variations were found in undiluted serum, but diluted serum showed a tendency to lower SILA values for women than for men, most pronounced before the 40th year.

Investigations using the rat epididymal fat method have shown that SILA rises after ingestion of glucose in normal subjects, both in undiluted and diluted serum (see tables 5 and 6). Following intravenous administration of glucose, a rise is likewise found in the SILA level in both undiluted and diluted serum (47, 159).

The result of the considerations just discussed is that the most likely values for biological SILA in venous serum from fasting normal persons are dependent to only a slight degree on the methods used for measuring SILA. In undiluted serum, a great number of investigators find mean values between 50 and 200 $\mu\text{U/ml}$, whereas higher values are found in diluted serum, the mean values varying between 150 and 200 $\mu\text{U/ml}$. Using all methods, a rise is found in the SILA level in both undiluted and diluted serum, after oral as well as intravenous administration of glucose.

It has not been fully elucidated why SILA determinations by the different modifications of the rat diaphragm method show such varying results. As mentioned previously (page 20), however, it seems reasonable to assume that technical details in the working out of the rat diaphragm method play a significant part. In this connection it might be emphasized that in none of the modifications of this method, is a "carrier protein" used in the standard insulin solutions. As a result, part of the insulin in these solutions may be adsorbed to the glass-ware, and the real insulin concentration in the standard insulin solutions will therefore be less than intended. Consequently, comparisons of the insulin-like-activity of plasma with the effect of the standard insulin solutions may result in too high values being determined for the insulin-like activity of plasma. It seems reasonable, therefore, to take the lowest normal values obtained by the rat diaphragm method, as being the most correct measure of SILA by this technique. Randle's own investigations (199) support the hypothesis that his results can hardly be a real measure of the SILA level in normal untreated plasma. For example, he found that a plasma which showed a SILA value of 13,000 μ U/ml by the rat diaphragm method, had no hypoglycaemic effect on alloxan-diabetic hypophysectomized rats. When insulin solutions with 2000 μ U/ml were injected into these animals, they reacted with a pronounced fall in blood sugar.

As a result of the considerations men-

tioned above, the normal values of SILA obtained by Shaw & Shuey (236) using Vallance Owen's modification of the rat diaphragm method must be considered the most correct values at present. These investigators added gelatine to the standard insulin solutions, and, as was anticipated, they found lower values of SILA than did other investigators, using Vallance Owen's modification without any addition of gelatine.

SILA determinations in peripheral venous serum using the rat epididymal fat method have given normal values which vary widely, although not so much as the results with the rat diaphragm method (tables 5 and 6). The cause of this variation has not been elucidated, but it is presumably due to technical details in connection with the preparation of the serum, and perhaps also with the details of the procedure. Lyngsøe has thus shown that by preparing serum from blood which is kept cooled to 4° C, lower SILA values are obtained than if the blood coagulates at room temperature (98, 152). Thawing frozen serum at room temperature also results in an increase in the SILA level (152), but not if the serum is thawed at 4° C (98). Renold's group made investigations using serum which had been prepared at room temperature and subsequently frozen, whereas Lyngsøe's investigations were made on fresh serum prepared at 4° C. It seems likely therefore, that the difference between the normal values in the two sets of results can be explained as a consequence of these conditions. Using a technique for the preparation

of serum which was similar to the one used by Renold's group, Lyngsoe (152) found normal values of an order of magnitude similar to those found by the group mentioned, whereas Gjedde (98), using serum prepared at 4° C, found SILA values which correspond to the values published in Lyngsoe's studies

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Most studies using the rat epididymal fat method do not give any details for the preparation of serum. It is therefore impossible to decide, whether the variation in normal values found by different workers merely results from the serum used being prepared in different ways. Reviewing the studies published, however, it is of interest to observe that all investigators using Renold's method

without modifications (66, 190, 237, 254) obtain almost similar normal values, whereas other groups who have worked out modifications of the rat epididymal fat method, obtain lower and identical normal values (19, 47, 98, 152, 153, 157, 198, 227).

A single material of normal subjects was examined with respect to variations in age and sex (153). No variations were found in undiluted serum, but diluted serum showed a tendency to lower SILA values for women than for men, most pronounced before the 40th year.

Investigations using the rat epididymal fat method have shown that SILA rises after ingestion of glucose in normal subjects, both in undiluted and diluted serum (see tables 5 and 6). Following intravenous administration of glucose, a rise is likewise found in the SILA level in both undiluted and diluted serum (47, 159).

The result of the considerations just discussed is that the most likely values for biological SILA in venous serum from fasting normal persons are dependent to only a slight degree on the methods used for measuring SILA. In undiluted serum, a great number of investigators find mean values between 50 and 200 $\mu\text{U/ml}$, whereas higher values are found in diluted serum, the mean values varying between 150 and 200 $\mu\text{U/ml}$. Using all methods, a rise is found in the SILA level in both undiluted and diluted serum, after oral as well as intravenous administration of glucose.

Immunologically active insulin in peripheral venous blood

Tables 7 and 8 show the studies made on the occurrence of immunologically active insulin in venous serum. In fasting serum, the immunological methods have given normal values amounting to about 20 μ U/ml, although Spellag & Goetz (243), using Goetz & Greenberg's method, obtained somewhat higher values. Studying the amount of SILA which is inhibited by the addition of anti-insulin ("suppressible SILA"), the majority of investigations by the rat epididymal fat method arrive at values which are about twice as high as those found by examination of immunologically active insulin using immunological methods. A single study, however, gives a mean value of 13 μ U/ml. It is of considerable interest that the investigations cited do not show values for suppressible SILA which are higher in di-

luted than in undiluted serum. As mentioned previously, the investigations using immunological methods do not show any dilution effect (113, 308).

All studies show a rise in both immunologically determined insulin and suppressible SILA following oral and intravenous administration of glucose (see tables 7 and 9). Considering the fact that the two methods for determining immunologically active insulin in serum are quite different in principle, it is surprising to find so little difference in the mean values quoted for the normal series examined. This strongly suggests identity between the part of the serum insulin determined by the immunological methods, and the part determined as suppressible SILA.

Since studies by the rat diaphragm method have shown that addition of anti-insulin to serum produces a complete inhibition of SILA, it is reasonable to assume that using this method, SILA

TABLE 7
Immunologically determined insulin in serum from normal subjects

Fasting values		Values after the oral administration of glucose				
		1 hour after glucose		1 1/2 hours after glucose		
μ U/ml	Mean value	μ U/ml	Mean value	μ U/ml	Mean value	
2-66	21(30)	18-342	139(30)	21-233	106(30)	Yalow & Berson (1960) ²⁴⁰
0-70	23(30)					Samols & Ryder (1961) ²⁴¹
2-63	19(100)					Samols & Marks (1963) ²⁴²
0-27	17(5)	20-100	49(5)			Hales & Randle (1963) ²⁴³
0-50	24(10)	25-170	80(10)	25-150	70(10)	Ruedi et al. (1963) ²⁴⁴
11-50	34(12)					Orskov (1963) ²⁴⁵
30-115	62(20)					Spellag & Cortz (1963) ²⁴⁶

The figures in brackets indicate the number of sera investigated

TABLE 8

Supplemental SILA and Non-supplemental SILA on normal study is

M	I	SIL A			Non-supplemental SILA		
		b		d	Gas		d
		ml	ml		ml	ml	
C	1/2	0-157	51	53-238	106		
	1/4	0-93	13-22	70-200	180(24)		
	1/8	0-40	13(5)	70-190	120(5)		
	1	0-60	30(5)	55-260	170(5)		
C	1/2	0-200	23(10)	80-670	270(10) ¹⁾		
	1/4	43(10)	180(10) ²⁾		164(10)		
	1/8	12-122	45(17)	33-440	131(17) ³⁾		
	1				0-210	82(17)	

Ran se et al (1961)¹⁾
Darg et al (1962)⁴⁾
doSan a i e al (1972)¹⁾
Sa ian et al (1963)¹⁾
Lyngsue & Lundbeck (1965)¹⁾

1) 101 mice after the oral administration of glucose (1/2) (normal values)

2) 101 mice after the intravenous administration of glucose (1/2) (normal values)

3) 101 mice after the intravenous administration of glucose (1/8) (normal values)

4) 101 mice after the intravenous administration of glucose (1/8) (normal values)

The figures in brackets indicate the number of animals used

Immunologically active insulin in peripheral venous blood

Tables 7 and 8 show the studies made on the occurrence of immunologically active insulin in venous serum. In fasting serum, the immunological methods have given normal values amounting to about 20 μ U/ml, although Spellacy & Goetz (243), using Goetz & Greenberg's method, obtained somewhat higher values. Studying the amount of SILA which is inhibited by the addition of anti-insulin ("suppressible SILA"), the majority of investigations by the rat epididymal fat method arrive at values which are about twice as high as those found by examination of immunologically active insulin using immunological methods. A single study, however, gives a mean value of 13 μ U/ml. It is of considerable interest that the investigations cited do not show values for suppressible SILA which are higher in di-

luted than in undiluted serum. As mentioned previously, the investigations using immunological methods do not show any dilution effect (113, 308).

All studies show a rise in both immunologically determined insulin and suppressible SILA following oral and intravenous administration of glucose (see tables 7 and 9). Considering the fact that the two methods for determining immunologically active insulin in serum are quite different in principle, it is surprising to find so little difference in the mean values quoted for the normal series examined. This strongly suggests identity between the part of the serum insulin determined by the immunological methods, and the part determined as suppressible SILA.

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Fasting values		Values after the oral administration of glucose				
		1 hour after glucose		1 1/2-2 hours after glucose		
μ U/ml	Mean value	μ U/ml	Mean value	μ U/ml	Mean value	
2-66	21(30)	18-342	139(30)	21-233	106(30)	Valow & Berson (1960) ²⁴⁰
0-70	23(30)					Samols & Ryder (1961) ²⁴¹
2-63	19(100)					Samols & Marks (1963) ²⁴²
0-27	17(5)	20-100	49(5)			Hales & Randle (1963) ²⁴³
0-50	24(10)	25-170	80(10)	23-150	70(10)	Ruedi et al. (1963) ²⁴⁴
11-50	33(12)					Orskov (1963) ²⁴⁵
30-115	62(20)					Spellacy & Goetz (1963) ²⁴⁶

The figures in brackets indicate the number of sera investigated

is a measure of the overall effect of the immunologically active insulin and insulin antagonists in serum. Using what must be considered the best modification of the rat diaphragm method (236), SILA values are found which correspond to the values found for suppressible SILA in most investigations by the rat epididymal fat method.

Immunologically inactive insulin in peripheral venous blood

As previously mentioned (chapter 2) it appears that immunologically inactive insulin in serum may be measured by three different methods: 1) By adding anti-insulin to serum and determining non-suppressible SILA by the rat epididymal fat method; 2) By examining ILA in serum and serum protein fractions after warm dialysis; 3) By treatment of serum with resin and elution of ILA from the resin. The second of these three methods, *viz.* warm dialysis of electrophoretically separated serum protein fractions, makes it possible to measure two fractions of immunologically inactive insulin in serum, the A fraction, which is localized to albumin- α_1 -globulin, and the B fraction, which is localized to β - γ -globulin. It is possible that resin treatment and determination of ILA in the eluate provides a measure of the immunologically inactive insulin localized to β - γ -globulin, but at present this problem remains unclarified.

The only quantitative studies published on the two fractions of immunologically inactive insulin in serum, the

A fraction and the B fraction, were made by electrophoretic separation of the serum protein fractions followed by warm dialysis. As previously mentioned (page 41), it is probable that both immunologically active and immunologically inactive insulin are determined by examining the albumin- α_1 -globulin fraction with this technique. Since the amount of immunologically active insulin is but a negligible part of the insulin content of this protein fraction, it is justifiable to ignore it, and to consider ILA in warm-dialyzed albumin α_1 globulin as a measure of the immunologically inactive insulin in this fraction.

It might be that part of the immunologically inactive insulin in serum could be isolated by extraction with acid alcohol. Studies of serum extracts by Grodsky, Forsham & Karam (103, 130), do not appear to support this assumption, as the values by this method were not significantly higher for immunologically determined insulin in the extract than those usually measured in untreated serum by the immunological methods (see table 9). Baird & Bornstein (16, 17) however, extracting serum by means of alcohol-toluene and determining ILA in the extract by the use of an *in vivo* method, found values that were considerably higher than those which may be demonstrated by a similar method in untreated serum (tables 5 and 9). It has also been shown that extract prepared in the same way contains considerable amounts of immunologically active insulin, determined by Arquilla & Stavitsky's method (see table 9). These

reasonable to suppose that all the methods determine only a greater or lesser portion of a large 'pool' of immunologically inactive insulin.

Several studies elucidate the changes in immunologically inactive insulin after ingestion of glucose. Using Baird & Bornstein's extraction technique, a rise in ILA was found in the extract (table 9). In warm dialyzed serum protein fractions a rise in ILA was found in the albumin- α_1 globulin fraction (table 10) which presumably reflects a rise in the immunologically inactive insulin in this protein fraction (157). In untreated serum, on the other hand, no rise was found in the amount of non suppressible SILA after ingestion of glucose (table 8). Non suppressible SILA determined in serum which has not been fractionated by electrophoresis presumably constitutes only a small part of the immunologically inactive insulin in serum. It seems reasonable, therefore, to assume that the results obtained by this technique reflect only to a slight degree the changes which occur in the amount of immunologically inactive insulin. From the available studies it seems highly probable that the amount of immunologically inactive insulin in peripheral venous blood will rise after ingestion of glucose presumably on account of a rise in the insulin in albumin- α_1 globulin.

A few investigators have examined immunologically inactive insulin in serum prior to and after intravenous administration of glucose. Burgi et al (table 8) found no change in non sup-

pressible SILA 120 minutes after administration. Anthoniades & co-workers (table 9) found a fall in the 'complex insulin', i.e. insulin which can be eluted from the resin, 10 minutes after administration of glucose. Lyngsoe & Lundbæk (table 8) likewise found no change in non suppressible SILA 10 minutes after glucose, just as ILA in the B fraction was unchanged (table 10). A fall was nevertheless found in ILA in the A fraction. In spite of this fall not being quite significant statistically, it is of considerable interest in the light of Anthoniades' results. These investigations by means of two different methods for determining the immunologically inactive insulin, suggest that a fall in insulin may occur soon after intravenous administration of glucose. Although Anthoniades' preliminary results seem to indicate that insulin which is adsorbed to resin migrates electrophoretically like β - γ globulin (see page 40), the possibility cannot be altogether excluded that the two series of investigations reflect a change in one and the same fraction of the immunologically inactive insulin.

SILA immunologically active and immunologically inactive insulin in blood from the portal vein, hepatic vein and peripheral arteries

In normal subjects, there are only a few investigations on the insulin content of blood not originating from a peripheral vein.

Humbel & co-workers (121) examining SILA (rat epididymal fat method) in the hepatic vein and the brachial

values for immunologically determined insulin in the serum extracts are considerably higher than values found by means of other immunological methods in untreated serum (see table 8) It must be considered likely, therefore, that immunologically inactive insulin in serum can be extracted by Baird & Bornstein's extraction method, but no studies are available which elucidate whether the extracted insulin derives from both or only one of the two previously described fractions of immunologically inactive insulin

In contrast to previous methods, it seems possible that Davidson's recently published technique of acid alcohol extraction of serum will permit extraction of immunologically inactive insulin (65) It is stated that up to 7000 μ U/ml serum has been demonstrated by this method, i.e. significantly more than corresponds to the amount of immunologically active insulin in serum Davidson's method employs prolonged dialysis of the acid

alcohol extract at 1° C, in contrast to other methods for insulin extraction, and preliminary studies suggest that this is the reason why Davidson's method gives higher values in the extract than the extraction methods so far used

Tables 8, 9 and 10 show studies elucidating the occurrence of immunologically inactive insulin in serum It appears from the tables that the normal values for non-suppressible SILA determined by the rat epididymal fat method, are somewhat lower than the ILA values which were found by Anthoniades' resin technique using the rat diaphragm method The normal values determined in warm dialyzed serum protein fractions, and found after extraction by Baird & Bornstein's method, are significantly higher than the ILA values found after resin treatment and as non suppressible SILA It is not known why the techniques mentioned for determining immunologically inactive insulin have given so varying results, but it seems

TABLE 10
ILA in warm dialyzed A fraction and B fraction from normal subjects

A fraction				B-fraction			
Fasting values		Values after the administration of glucose		Fasting values		Values after the administration of glucose	
μ U/ml	Mean value	μ U/ml	Mean value	μ U/ml	Mean value	μ U/ml	Mean value
390-7200	1900(18)	300-10800	3142(11)	560-13000	2820(18)	760-12400	3685(11) ¹⁾
260-5800	1740(18)	200-4800	1300(15)	320-6100	2530(18)	500-4200	2270(15) ²⁾

¹⁾ Lyngsoe (1964)¹⁵⁹ The normal subjects were investigated in the fasting state and 60 minutes after the oral administration of glucose

²⁾ Lyngsoe & Lundbæk (1965)¹⁵⁸ The normal subjects were investigated in the fasting state and 10 minutes after the intravenous administration of glucose

The figures in brackets indicate the number of sera investigated

found values between 5 and 12 $\mu\text{U}/\text{ml}$ (mean value 9 $\mu\text{U}/\text{ml}$) in 4 subjects. These values are somewhat lower than the values which are usually found by Yalow & Berson's method for immunologically-determined insulin in peripheral venous plasma (see table 7)

SERUM INSULIN IN EXPERIMENTAL ANIMALS

SIL 1, immunologically active and immunologically inactive insulin in peripheral venous blood

It appears from table 11 that the mean SILA values found by various investigators in fasting experimental animals corresponded in order of magnitude to those found in normal subjects by the same methods. There is only one exception to this rule, Steinke & co-workers (257) finding of an average SILA value of 106 $\mu\text{U}/\text{ml}$ (rat epididymal fat method) in undiluted cat serum, whereas an average value of 290 $\mu\text{U}/\text{ml}$ was found in fasting normal subjects (table 5)

Studies on the occurrence of immunologically active insulin in serum from experimental animals indicate that rats have higher fasting values of immunologically-determined insulin than humans (116-175). On the other hand values of immunologically-determined insulin in fasting dogs were found to correspond to the values in humans.

The amount of immunologically active insulin in blood from experimental animals has been elucidated both by

extraction studies and by determination of non suppressible SILA by the rat epididymal fat method. These investigations, too, show values in fasting experimental animals which correspond to the findings in human blood (tables 8 and 9)

Samaan et al (226) found a rise in both suppressible and non suppressible SILA after intravenous administration of glucose in three dogs. This finding differs from corresponding studies on normal subjects, which all showed an unchanged non suppressible SILA level after intravenous administration of glucose (table 8). As the study comprised only a few animals, however, it is not possible to conclude from it that immunologically inactive insulin in humans and in dogs reacts differently after intravenous administration of glucose.

SILA immunologically active and immunologically inactive insulin in blood from the pancreatic vein and the hepatic vein

Studies on the insulin content of blood from the pancreatic vein and the hepatic vein have only been made in dogs. Using the rat diaphragm method and the rat epididymal fat method, several investigators have shown that the SILA level in blood from the portal vein and the pancreatic vein is higher than the SILA level in peripheral venous blood and blood from the hepatic vein. The same investigators showed that SILA in blood from the pancreatic vein rises after the administration of glucose (74, 170)

vein, found the highest SILA level in the hepatic vein

Using Vallance-Owen's modification of the rat diaphragm method, Baird & Farquhar (18) found that plasma from the portal vein in the new-born had a mean SILA value of 200 μ U/ml. Five minutes after intravenous administration of glucose there was a fall in the SILA level to an average value of 72 μ U/ml¹

Anthoniades et al (11), using the rat diaphragm method, examined SILA and ILA in resin eluates of blood from the portal vein. In fasting normal subjects, SILA varied between 200 and 2000 μ U/ml, whereas the mean value for ILA in the resin eluates corresponded to 50 μ U/ml. After intravenous administration of glucose, SILA rose in 3 out of 4 normal subjects, while the ILA values in the resin eluates remained unchanged.

Using the same technique, the same investigators (12) examined blood from the hepatic vein. In two fasting normal subjects, SILA was found to be 50 and 450 μ U/ml, respectively, whereas the values for ILA in the resin eluates corresponded to 550 and 500 μ U/ml serum. Following intravenous administration of glucose, SILA rose while ILA in the resin eluates fell in both the normal subjects.

Samols & Ryder (230), in an excellent study, used Yalow & Berson's method for investigation of immunologically-determined insulin in the hepatic vein. An average of 24 μ U/ml was found in 7 subjects with porto-caval anastomosis or thrombosis of the portal vein. This

value is slightly higher than the mean value found by Samols & Ryder in peripheral venous blood from fasting subjects. In the same study, immunologically determined insulin was measured in blood from the femoral artery. The mean value for immunologically determined insulin in serum from the femoral artery was found to be 26 μ U/ml, and higher than the normal value in serum from peripheral veins. Samols & Ryder examined the concentration of both immunologically-determined endogenous insulin and of immunologically-determined exogenous ox insulin in the hepatic vein, femoral vein and peripheral arteries. They concluded from their results that an uptake of both endogenous and exogenous insulin occurred in both the liver and the lower extremities. This conclusion is correct, provided that no change takes place from immunologically active to immunologically inactive insulin in the liver or the lower extremities, but investigations made by Sammaan & co-workers (226, 227) seem to indicate that this possibility can hardly be excluded.

Rizzo & co-workers (220) have compared SILA (the rat diaphragm method) in the femoral artery and vein. They found a higher SILA level in arterial plasma than in venous plasma, after ingestion of glucose. Ruedi et al (225), using the rat epididymal fat method, found corresponding conditions in fasting normal subjects.

Rabinowitz & Zierler (193) examined arterial plasma from fasting normal subjects by Yalow & Berson's method. They

found values between 5 and 12 $\mu\text{U/ml}$ (mean value 9 $\mu\text{U/ml}$) in 4 subjects. These values are somewhat lower than the values which are usually found by Yalow & Berson's method for immunologically-determined insulin in peripheral venous plasma (see table 7).

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TABLE 11
Determination of insulin in peripheral venous blood from experimental animals

	Method	Mean fasting value $\mu\text{U/ml}$	
Rat	Randle's modification of the rat diaphragm method	14600	v Holt et al (1957) ¹¹³
	Own immunologic method	121(15)	Hales & Kennedy (1964) ¹¹⁴
	Own immunologic method	104	Morgan & Lazarow (1963) ¹¹⁵
	Acid ethanol extraction + Arquilla & Stavitsky's immunologic method	17400(10)	Pellegrini et al (1958) ¹¹⁶
	Baird & Bornstein extraction + Arquilla & Stavitsky's immunologic method	1800-18000	Arquilla et al (1960) ¹¹⁴
	Randle's modification of the rat diaphragm method	16000(5)	Randle & Young (1956) ¹⁰⁸
	Rat epididymal fat method	125(22) diluted $\frac{1}{8}$	Dossy (1963) ¹¹⁸
Dog	Hypophysectomized adrenodemic dullated rats	100	Gellhorn et al (1941) ¹¹⁹
	Own modification of the rat diaphragm method	270(10) diluted $\frac{1}{30}$	Takeuchi et al (1957) ¹²⁰
	do	288(8) diluted $\frac{1}{30}$	Takeuchi et al (1961) ¹²¹
	Vallance Owens modification of the rat diaphragm method	64(3)	Candela et al (1958) ¹¹⁸
	do	160(13) arterial plasma	Metz (1960) ¹²²
	Rat epididymal fat method	610(9) diluted $\frac{1}{8}$	Egdahl & Goldberg (1962) ¹²³
	do	48(3) suppressible SILA	Samaan et al (1962) ¹²⁴
Cat	Randle's modification of the rat diaphragm method	2400(5)	Randle & Young (1956) ¹⁰⁸
	Vallance Owens modification of the rat diaphragm method	119(5)	Vallance Owen & Lukens (1957) ¹²⁵
	Rat epididymal fat method	106(6)	Steinke et al (1962) ¹²⁷
	do	99(3) non suppressible SILA diluted $\frac{1}{8}$	do
Fig	Rat epididymal fat method	111(5)	Lyngsøe (1962) ¹²⁸
	do	510(5) diluted $\frac{1}{8}$	do
Cow	Baird & Bornstein extraction + own modification of the rat diaphragm method	260(12)	Cunningham (1962) ¹²⁴
Sheep	do	300(12)	Cunningham (1962) ¹²⁴
Monkey	Rat epididymal fat method	97(11) undiluted	Ball & Knobel (1963) ¹²⁹
	do	198(11) diluted $\frac{1}{8}$	do
	do	795(11) diluted $\frac{1}{16}$	do

The figures in brackets indicate the number of sera investigated

187, 226, 234) Metz (170), using the rat diaphragm method, showed that there is a proportionality between the serum glucose level and the SILA values in blood from the pancreatic vein.

Samaan & co-workers (226) have made a thorough investigation of the changes in suppressible and non-suppressible SILA in blood from the pancreatic vein, hepatic vein and femoral vein in dogs following the administration of glucose. They found that the values for suppressible SILA in the pancreaticoduodenal vein were significantly higher than the values for non-suppressible SILA in both fasting animals and after administration of glucose. Both before and after administration of glucose much higher values of suppressible SILA were found in the pancreaticoduodenal vein than in the hepatic vein and femoral vein. The values for non-suppressible SILA were identical in the three types of venous blood while fasting. After administration of glucose a rise in non-suppressible SILA was seen in all three veins but the rise was most pronounced in the femoral vein.

In a subsequent study Samaan & co-workers (229) showed that on infusion of large quantities of dog and ox insulin into the portal vein in pancreatized dogs there was a moderate rise in non-suppressible SILA and a considerable rise in suppressible SILA in the hepatic vein. The same investigators found a fall in non-suppressible SILA in peripheral venous blood on infusion of insulin into the femoral vein in two pancreatectomized dogs, whose liver had

been excluded from the blood circulation. On these findings, Samaan & co-workers proposed the hypothesis that non-suppressible SILA is formed in the liver. This hypothesis agrees with the finding of reduced values of non-suppressible SILA in peripheral venous blood, in patients with cirrhosis of the liver (227).

Burgi & co-workers (48), perfusing isolated rat liver with rat insulin, were unable to demonstrate any rise in non-suppressible SILA during the perfusion.

DISCUSSION

It appears from the foregoing that a number of studies of peripheral venous blood elucidate the changes in immunologically active and immunologically inactive serum insulin after administration of glucose. These studies have shown that the immunologically active insulin, determined as suppressible SILA or immunologically-determined insulin, increases after both intravenous and oral administration of glucose. Most studies on peripheral venous blood suggest that ingestion of glucose is followed by a rise in immunologically inactive insulin, and this probably reflects a change in the A fraction. A few studies suggest that the immunologically inactive serum insulin will fall soon after intravenous administration of glucose, possibly reflecting a fall in the A fraction. No change in the B fraction has been observed, either after intravenous or oral administration of glucose. These findings show that the different forms and frac-

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Pig	Rat epididymal fat method	111(5)	Lyngsoe (1962) ¹³⁸
	do	510(5) diluted 1/6	
Cow	Baird & Bornstein extraction + own modification of the rat diaphragm method	260(12)	Cunningham (1962) ¹⁴⁴
	do	300(12)	Cunningham (1962) ¹⁴⁴
Monkey	Rat epididymal fat method	97(11) undiluted	Ball & Knobel (1963) ¹⁴⁰
	do	198(11) diluted 1/5	
	do	790(11) diluted 1/10	

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tions of serum insulin react differently when the blood sugar level changes, so that it is reasonable to ascribe different physiological significance to them

Studies in both man and experimental animal suggest that the concentration of immunologically active insulin is greater in the pancreatic vein and the portal vein than elsewhere in the circulation (see pages 62, 63, and 65) Examination of pancreatic venous blood has also shown that SILA measured by the rat diaphragm method rises in proportion to the blood sugar level (170) These investigations agree with the assumption that immunologically active insulin is formed in the pancreas, and that the production is regulated by the blood sugar level

Studies in man indicate that the concentration of immunologically active insulin is higher in the peripheral arteries than in the peripheral veins (see page 62) No investigations are available which elucidate whether this is due to a peripheral uptake of insulin, or to a change from an immunologically active

form of insulin to an immunologically inactive form

A few studies seem to suggest that the concentrations of non suppressible SILA and "complex insulin" (i.e. insulin which can be eluted from resin), respectively, are lower in the portal vein than in the hepatic vein (see page 62 and page 65) These findings support the assumption that immunologically inactive insulin is formed in the liver, a concept compatible with the investigations on liver "insulin uptake" by Samols & Ryder (see page 62)

In all the studies of serum from the portal vein and the hepatic vein, the immunologically inactive insulin has been determined by methods which show only a small proportion of this form of insulin, and which do not distinguish the A and B fractions In view of this, it must be concluded that for the time being, these studies do not permit a decision as to the site of formation of immunologically inactive insulin in the normal organism

CHAPTER 5

INSULIN CONTENT OF BLOOD FROM PATIENTS WITH DIABETES MELLITUS

Most studies on serum insulin in patients with diabetes mellitus show serum levels which vary according to the type of diabetes mellitus involved. These studies are difficult to compare as the patient series are often classified on the basis of different clinical characteristics and in many cases the clinical information is so scanty that the patients cannot be grouped in any other way than that followed in the original publication. In the present study, an attempt has been made to systematize these investigations by subdividing patients with diabetes mellitus into two groups: those with acidosis and those without acidosis. The latter group has been subdivided further into different types.

On reviewing the studies made on poorly regulated diabetic patients, it was found that some of the patients had been examined when they had high blood sugar level and considerable ketonuria but that information on serum pH or serum bicarbonate was lacking. It was decided to regard these patients as not having acidosis unless it was stated that the patients were in diabetic acidosis, precoma, or coma.

In the present survey of serum insulin investigations on non-acidotic diabetic patients, an attempt has been made to classify the patients examined into 3 groups: 1) juvenile diabetics, comprising all young patients, together with those older diabetic patients whose disease—as in the case of the young diabetics—is accompanied by heavy glycosuria and a tendency to ketosis. 2) Older non-obese diabetics. 3) Older obese diabetics. In this grouping, the designations 'juvenile' and 'older' refer to patient age on diagnosis of the diabetes mellitus. On this classification, the group 'older non-obese diabetics' includes only older patients with a 'mild' type of diabetes mellitus, i.e. without ketonuria and without pronounced glycosuria. Many investigations on older diabetics without obesity, however, state nothing as to the patient's tendency to ketosis. In the present study, it has been chosen to classify such patients as 'older non-obese diabetics', since patients with a tendency to ketosis constitute a minority within the group of older diabetics without obesity. On similar grounds, studies on obese diabetics whose

age is not stated have been grouped together with the studies on "older obese diabetics"

It will appear from the above that these attempts at grouping the patients who are reported in the literature, are far from certain. It is regrettable that so many of the publications on plasma insulin do not meet the simplest requirements for clinical description of patient material. In many otherwise excellent and extensive studies, therefore, it is only with difficulty and considerable uncertainty that the results presented can be included in the discussion on the pathophysiology of diabetes mellitus.

SERUM INSULIN IN NON ACIDOTIC PATIENTS WITH DIABETES MELLITUS

SILA in peripheral venous blood

Investigations by means of the ADH-rat method, and most investigations by the rat diaphragm method, show either no SILA or low SILA values in patients with juvenile diabetes mellitus. It appears from table 12 that those investigators who have been unable to demonstrate SILA in serum from juvenile diabetics, have examined undiluted serum, whereas in the remaining studies, SILA was examined in diluted serum. The difference found between determinations on diluted and undiluted serum is probably due to the presence of an insulin antagonist which is without any effect in diluted serum (276), although at present it is not possible to establish which of the insulin antagonists described is responsible.

Examination of SILA in juvenile diabetics by means of the rat epididymal fat method shows a different pattern. Normal fasting SILA values are found in some studies (78, 154, 155), while elevated values have been demonstrated in other studies (66, 189, 253, 254, 255). The cause of these divergencies is unknown. The increased SILA in the juvenile diabetics studied by Daweke (66) may well be due to exogenous insulin, but this cannot be the reason for the high SILA values in Steinke's investigations (253, 254, 255) as these latter determinations were made exclusively on untreated patients. More recent investigations, however, seem to indicate that fasting serum from juvenile diabetics contains normal quantities of immunologically active insulin (see page 70), but increased quantities of immunologically inactive insulin (see page 77). It might be assumed, therefore, that those modifications of the rat epididymal fat method used by Steinke & Daweke, determine immunologically inactive serum insulin to a greater degree than in the case with the modification used by Lyngsøe & co-workers. Support for this assumption is gained from the fact that the normal values for fasting SILA found by the two first-mentioned investigators, are considerably higher than the values published by Lyngsøe (see tables 5 and 6).

Lyngsøe (154) found a rise in SILA in juvenile diabetics after administration of glucose. In contrast to this Daweke (66) found a fall in SILA in this type of patient after administration of glu-

case All the juvenile diabetics in Dawke's study were given a slowly resorbable insulin 24 hours prior to the SILA investigation, but only 25 % of the patients in Lyngsø's study received this. The reason for the difference between the two investigations, therefore, may be that Dawke, in contrast to Lyngsø, determined the exogenous insulin in serum.

Using the rat diaphragm method, normal fasting SILA values are found in older non-obese diabetics (218). Seltzer & Smith (235), examining this group of patients found a difference between Tolbutamide responders and Non Tolbutamide responders. If these two groups are considered as one, however, this study also finds normal SILA values in older non-obese diabetics. Most investigations by means of the rat epididymal fat method show normal or low normal fasting SILA values in this type of patient (66, 67, 154, 155). In older non obese diabetics there is a rise in SILA following administration of glucose.

In most studies with both the rat diaphragm method and the rat epididymal fat method an elevated fasting SILA level is found in older obese diabetics. Nevertheless, using the same method or the ADHA rat method a few investigators have found normal SILA values in this type of patient (40, 66, 67, 218). Eskjær Jensen & co-workers (78) however found that the SILA level in older obese diabetic patients is dependent on the patient's intake of food. In the study cited high SILA values were found in obese diabetics who received

a normal diet, but after a brief period of diet with reduced carbohydrate content, SILA fell to normal values in those patients. A possible reason why the investigations cited gave diverging results is that some patient groups were examined during dietary treatment, whereas other groups were examined while receiving a normal diet. In line with this assumption Lyngsø's investigations (154, 155) were carried out on patients who received a normal diet, or on a mixed population of diabetic patients on diet and diabetic patients not on diet, whereas Dawke's (66, 67) and Rizzo's investigations (218) were performed in patients under dietary treatment. The other publications on older obese diabetic patients lack information on the patients' caloric intake during the period of investigation.

Most investigators found a rise in SILA in older obese diabetic patients after administration of glucose. Phear (191) however, found a fall in the SILA level.

Steinke & co-workers (254), using the rat epididymal fat method, examined SILA in a great number of older diabetic patients. Their study demonstrated elevated SILA values in this group, but no information is available on the weight of the patients.

Immunologically active insulin in peripheral venous blood

Most studies on serum from juvenile diabetics (tables 13 and 14) show reduced or normal fasting values for im-

TABLE 13

Immunologically determined insulin in the serum from non acidotic patients with diabetes mellitus

Diabetics investigated				Fasting mean value μ U/ml	Mean value after the administration of glucose μ U/ml	In crease after glu cose	
	Age of onset	Mean value fasting blood sugar mg %	Insulin uria				
Untreated	Older	110		27(38) high normal	156(38) ¹⁾ normal	+	Yalow & Berson (1960) ¹⁰⁸
Treated	Juvenile	318		7(10) decreased	4(3) ¹⁾ decreased	0	Berson & Yalow (1962) ¹¹
Untreated		190 318	~ +	37(17) increased 25(4) normal	58(17) ¹⁾ normal	+	Hales & Randle (1963) ¹¹¹

¹⁾ 60 minutes after the oral administration of glucose

The figures in brackets indicate the number of sera investigated

immunologically-determined insulin and suppressible SILA. Only one study shows elevated suppressible SILA in these patients (85). The reason for the divergent results obtained by these experienced investigators is not known. All the patient series are small, however, and the method for determination of suppressible SILA is encumbered by a very pronounced uncertainty. If all the series investigated are considered under one, it seems most likely that juvenile diabetic patients have normal or low normal values for immunologically active insulin in serum.

Neither oral nor intravenous administration of glucose was followed by an increase in this form of insulin, in any of the published investigations on immunologically active insulin in serum from juvenile diabetics.

In older, non-obese diabetic patients, reduced or normal suppressible SILA,

respectively, was found in the fasting state, while no rise was found after administration of glucose (table 14).

Samaan & Fraser (228) found a normal fasting suppressible SILA in older obese diabetics, whereas Lyngsøe & Lundbæk (159) found elevated values. These latter investigators observed no change in immunological SILA shortly after intravenous administration of glucose, whereas Samaan & Fraser recorded a rise 120 minutes after oral administration of glucose.

Several investigators have recorded suppressible SILA (85) and immunologically-determined insulin (114, 308) in fairly large series of older diabetics. In the publications cited, there is no information on the patients' weight. These investigators all found elevated or high normal fasting values, and a rise in immunologically determined insulin after oral administration of glucose. The rise

TABLE 14
Suppressible and non suppressible SII in untreated newly diagnosed non-acetotic patients with diabetes mellitus

Diabetic evaluation			Suppressed SII, μ mol/l		Non-suppressed SII, μ mol/l	
Age of onset	Glucose in blood mg	Ketone in urine	fasting glucose mg	fasting insulin μ U/ml	fasting glucose mg	fasting insulin μ U/ml
6-35	191	+	19(5) decreased	10(5) ¹⁾ decreased	48(5) decreased	40(5) ¹⁾ decreased
36-61	211	-	15(5) decreased	1(5) ¹⁾ decreased	91(5) decreased	90(5) ¹⁾ decreased
26-67	216	-	42(9) normal	80(9) ¹⁾ normal	203(9)	180(9) ¹⁾ high normal
Juvenile	299		41(10) increased		99(10) low normal	
Older	165		49(18) high normal		130(18) low normal	
7-37	245	±	75(5) high normal	90(4) ¹⁾ low normal	110(4) normal	87(4) ¹⁾ normal
42-63	180	-	43(5) normal	106(5) ¹⁾ low normal	130(5) high normal	80(5) ¹⁾ normal
42-69	212	-	80(8) increased	101(8) ¹⁾ low normal	133(8) increased	101(8) ¹⁾ high normal

Samaan & Fraser (1963)¹⁾

Troesch et al (1963)¹⁾

Lingsoe & Lundberg (1964)¹⁾

¹⁾ investigated 120 minutes after the oral administration of glucose
²⁾ investigated 10 minutes after the intravenous administration of glucose
 The figures in brackets indicate the number of sera investigated

TABLE 15
Immunologically determined insulin and ILA in serum extracts from non acidotic patients with diabetes mellitus

Method	Diabetics investigated			Fasting mean value μ U/ml	
	Age of onset	Mean value of fasting blood sugar mg%	keton urine		
Baird & Bornstein extraction + A D A rats	17-25 35-63			1000(3) low normal 4900(3) high normal	Baird & Bornstein (1959) ¹⁷
do	Juvenile Older			0(7) decreased (14) normal	Bornstein & Hyde (1959) ¹⁸
Baird & Bornstein extraction + rat diaphragm	16-63	390	-	(10)*	Taylor (1963) ¹¹¹
Baird & Bornstein extraction + Arquilla & Slavitsky	Older	275	±	4500(4) decreased	Rodari et al (1961) ¹¹²
Baird & Bornstein extraction + Arquilla & Slavitsky	Older	165	-	18900(10) high normal	
Baird & Bornstein extraction + Arquilla & Slavitsky	35-82 25-66	180 300	- +	19840(13) high normal 7410(6) decreased	Rizzo et al (1961) ¹¹³
Rein extraction + rat diaphragm	Untreated	220		320(6) normal	Antoniadou et al (1961) [•] 1962 ¹¹⁴

*) indicates that ILA has been found
The figures in brackets indicate the number of sera investigated

after the administration of glucose occurred later than in normal subjects, and reached a higher level. This result is not incompatible with the investigations just cited, performed on older obese diabetic patients nor with investigations elucidating immunologically active serum insulin in older non-obese diabetics (table 14). All the studies on older diabetics are thus in accordance with the assumption that the immunologically active serum insulin in these patients increases after administration of glucose but that this rise occurs more slowly than in normal subjects.

Immunologically inactive insulin in peripheral venous blood

As previously discussed (see page 48), it is probable that a measure of the amount of immunologically inactive insulin in serum can be obtained by four different methods: 1) determination of non-suppressible SIIA by the rat epi-

didymal fat method, 2) determination of ILA in warm dialyzed serum protein fractions, 3) resin treatment of serum and determination of ILA in the resin eluate, and 4) extraction of serum by Baird & Bornstein's technique. All these methods appear to have been used for examining the insulin content in serum from diabetics (tables 14, 15, and 16).

The distribution of ILA in electrophoretically separated serum protein fractions from patients with diabetes mellitus has been studied in two investigations. Taylor (262) examined 6 patients with untreated diabetes mellitus and ketonuria. On separation of serum on treated cellulose acetate and determination of ILA by the rat diaphragm method, he found ILA in albumin in 4 patients, and ILA in β - γ globulin in 3 patients. The examination of β - γ globulin was incomplete in two patients, and in one patient, no ILA was found in any of the fractions examined. In one patient, ILA was found

TABLE 16

ILA in warm-dialyzed A and B fraction from untreated acutely diagnosed non-acidotic patients with diabetes mellitus
Lyngsø & Lundbek (1955)¹²⁰

Age	ILA in fasting blood serum	Insulin	A-fraction μ U/ml		B-fraction μ U/ml	
			Mean fasting value	Mean value 10 min after v glucose	Mean fasting value	Mean value 10 min after v glucose
7-37	245	±	7840(5) high normal	2173(3) high normal	4192(5) high normal	3333(3) high normal
42-63 non-obese	180	—	1060(5) normal	820(5) normal	1725(5) normal	1480(2) normal
42-69 obese	212	—	1500(8) normal	1576(7) normal	3933(8) increased	2610(7) increased

The figures in brackets indicate the number of sera investigated

in a globulin, in which ILA cannot be demonstrated in normal subjects by the technique employed Taylor concludes, however, that the qualitative distribution of ILA in diabetic patients does not differ from the distribution found in normal subjects

Lyngsøe & Lundbæk (159) examined electrophoretically separated serum protein fractions from 2 juvenile, 2 older non-obese and 2 older obese untreated diabetic patients, without finding any abnormal distribution of ILA in these patients

Although these investigations are too limited to exclude the possibility that abnormal fractions of immunologically inactive insulin may be present in a few diabetics, this does not seem to be the case in the great majority of patients

Quantitative examinations of immunologically inactive insulin in juvenile diabetics have given very diverging results Thus, reduced or normal values have been found for non-suppressible SILA (table 14), increased ILA has been found in both the A and the B fractions (table 16), and no or low ILA has been found in serum extracts prepared by Baird & Bornstein's technique (table 15) It is not known why ILA cannot be demonstrated in the majority of juvenile diabetics by the last-mentioned technique, but in view of the fact that by other methods, immunologically inactive insulin could be demonstrated in serum from untreated juvenile diabetics, it must be considered less likely that the examinations by Baird & Bornstein's technique give a correct measure

of the amount of immunologically inactive insulin in these patients A considerably smaller fraction of the immunologically inactive ILA is determined by measurements of non-suppressible SILA than by examination of ILA in warm dialyzed A and B fractions, and it must be considered likely, therefore, that the latter technique gives the most correct measure of immunologically inactive insulin It may thus be assumed that juvenile diabetics have an increased amount of immunologically inactive insulin, corresponding to both the A and the B fractions

In older non-obese diabetics, low or normal values for non-suppressible SILA are found (table 14), as well as low values for immunologically-determined insulin in serum extracted by Baird & Bornstein's method (table 15), whereas ILA in warm dialyzed serum protein fractions is of the same order of magnitude as in normal subjects (table 16) These investigations all agree with the assumption that serum from older, non-obese diabetic patients contains reduced or normal quantities of immunologically inactive insulin

Elevated or high normal values for non-suppressible SILA were demonstrated in older obese diabetics (table 14) High normal values for immunologically-determined insulin were found on extraction by Baird & Bornstein's method (table 15), and an increase in ILA in the B fraction was found in a single investigation (table 16) These investigations thus seem to indicate that obese older diabetics have increased amounts

of immunologically inactive insulin in serum, and it is likely that this is an expression of an increase in the immunologically inactive insulin in the B fraction

Anthoniades & co-workers (9, 12), examining a number of diabetics whose characteristics were not specified, showed that these patients have normal fasting amounts of 'complex insulin' (i.e. ILA which can be eluted from resin). While these investigators could demonstrate a fall in complex insulin after intravenous glucose administration in normal subjects, they did not find a corresponding fall in patients with diabetes mellitus. This result which is of considerable interest, has not yet been confirmed by other investigators.

SERUM INSULIN IN PATIENTS IN DIABETIC ACIDOSIS

Low SILA values are reported when diluted serum from patients with diabetic acidosis is examined by the rat diaphragm method. In a single study (16) in which the serum dilution is not stated, no SILA was found at all. Normal SILA was found in both diluted and undiluted serum by the rat epididymal fat method (table 17).

Normal values were found on examining ILA in serum extracts prepared by Baird & Bornstein's method and on determining non suppressible SILA. This suggests that serum from patients in diabetic acidosis contains normal quantities of immunologically inactive insulin.

It is not possible to decide whether serum from acidotic diabetics contains normal or reduced amounts of immunologically active insulin, as all the investigations elucidating the occurrence of this were made by the biological methods. In spite of the fact that diluted serum has been used in these studies, it is difficult to exclude the possibility that the serum insulin antagonist present in undiluted serum from acidotic diabetics but which apparently disappears after dilution (see page 44), may inhibit the action of the excessively small amount of immunologically active insulin present in diluted serum. This assumption is in agreement with investigations on suppressible SILA made by Froesch & co-workers (85). These investigators were thus unable to demonstrate suppressible SILA in serum from acidotic diabetics, in spite of finding elevated values in both juvenile and older non acidotic diabetics. Froesch & co-workers' investigation may of course be interpreted as a consequence of a changed insulin metabolism in patients in diabetic acidosis, but so far, no other studies have suggested such a changed insulin metabolism in this condition. It may therefore be reasonable to assume that those changes which take place in the properties of the serum insulin in diabetics who develop acidosis, are due to the presence of a special serum insulin antagonist.

DISCUSSION

As will appear from the preceding discussion attempts to determine insulin

in α_2 -globulin, in which ILA cannot be demonstrated in normal subjects by the technique employed Taylor concludes, however, that the qualitative distribution of ILA in diabetic patients does not differ from the distribution found in normal subjects

Lyngsøe & Lundbæk (159) examined electrophoretically separated serum protein fractions from 2 juvenile, 2 older non-obese and 2 older obese untreated diabetic patients, without finding any abnormal distribution of ILA in these patients

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In older non-obese diabetics, low or normal values for non-suppressible SILA are found (table 14), as well as low values for immunologically-determined insulin in serum extracted by Baird & Bornstein's method (table 15), whereas ILA in warm dialyzed serum protein fractions is of the same order of magnitude as in normal subjects (table 16). These investigations all agree with the assumption that serum from older, non-obese diabetic patients contains reduced or normal quantities of immunologically inactive insulin.

Elevated or high normal values for non-suppressible SILA were demonstrated in older obese diabetics (table 14). High normal values for immunologically-determined insulin were found on extraction by Baird & Bornstein's method (table 15), and an increase in ILA in the B fraction was found in a single investigation (table 16). These investigations thus seem to indicate that obese older diabetics have increased amounts

lasting blood sugar values in the patients examined. As the pancreatic production of the immunologically active insulin (determined by means of the rat diaphragm method) is presumed to be proportional to the blood sugar level (170), it may be anticipated that increased values for immunologically active insulin in peripheral venous blood will correspond to increased fasting blood sugar. This assumption is supported by Seltzer's (233) observation, that continuous intravenous perfusion of glucose into normal subjects results in a considerable increase in SILA (the rat diaphragm method) in peripheral venous blood in spite of a very moderate rise in the blood sugar values. The demonstration of normal values of immunologically active insulin in diabetics with considerably increased fasting blood sugar must thus mean that relative to the increased fasting blood sugar, these values of insulin are reduced.

Determination of ILA in the A and B fractions from juvenile diabetics showed elevated values in both fractions (see page 74). This agrees with the finding of Steinke & co-workers that this type of diabetic has elevated SILA values, determined by the modification of the rat epididymal fat method used by these investigators (see page 68).

Thus the investigations made so far seem to indicate that in peripheral venous blood juvenile diabetics have relatively reduced values of immunologically active insulin but elevated values of immunologically inactive insulin both in the A and in the B fraction. Know-

ledge of the insulin metabolism in the normal and diabetic organism, however, is very limited. The only investigations available in diabetics elucidate the two forms of insulin in the portal vein of two patients whose characteristic data were not further specified (11). Our present knowledge of the serum insulin in peripheral venous blood from diabetics thus does not permit us to determine the locus of the defect in the insulin metabolism of these patients. It must be considered established, however, that juvenile diabetics have an insulin production, in spite of the fact that no insulin has been demonstrated in an acid alcohol extract of pancreas from these patients (206, 301). After glucose administration, however, there is no rise in immunologically active insulin in peripheral venous blood, so it must be assumed either that the pancreas is unable to increase the production of this type of insulin, or that the immunologically active insulin is used up or is transformed into immunologically inactive insulin peripheral to the pancreas. The fact that peripheral venous serum in juvenile diabetics contains an increased amount of immunologically inactive insulin might perhaps tell in favour of the last mentioned hypothesis, this is also compatible with the fact that there is an increase in SILA (determined by the rat epididymal fat method) after ingestion of glucose by juvenile diabetics (see page 68) as this rise may be due to immunologically inactive insulin.

What part the abnormality of insulin metabolism in juvenile diabetics plays

TABLE 17
Serum insulin in patients in diabetic acidosis

Method	Diabetes investigated	Total CO ₂ meq/l	Dilution	Mean fasting values μ U/ml	
Rat diaphragm do	Diabetic coma Diabetic coma and precoma	<15	$\frac{1}{8}$ $\frac{1}{10}$	(4) decreased 106(11) decreased	Groen et al (1952) ¹⁰⁸ Willebrands (1960) ¹¹¹
do Baird & Born stein extraction + Rat dia phragm	Diabetic coma do			0(7) decreased (7) normal	Baird & Bornstein (1957) ¹¹² do
Rat epididymal fat do	Diabetic coma		$\frac{1}{10}$	(8) low normal	Beigelman (1959) ¹¹³
do		<15	undil $\frac{1}{6}$	95(5) normal 310(5) high normal	Lyngsoe (1962) ¹¹⁴
do	Diabetic coma		$\frac{1}{8}$	Suppressible SILA 0(7) low normal Non suppress SILA 186(7) normal	Froesch et al (1963) ¹¹⁵

The figures in brackets indicate the number of sera investigated

in peripheral venous serum from patients with diabetes mellitus have shown so far that insulin is present in serum from all types of diabetics. This insulin, however, differs in several respects from insulin found in normal subjects. It has further been shown that the abnormalities demonstrated in the serum insulin are probably different in the different types of diabetic patients.

Juvenile diabetics

Examining juvenile diabetics, reduced or normal values were found for immunologically-determined insulin (see page 70), for SILA in diluted serum

determined by the rat diaphragm method (see page 68), and for suppressible SILA (see page 70). In contrast to normal subjects, no increase in suppressible SILA or immunologically determined insulin was found in these patients after oral and intravenous administration of glucose (see page 70). These findings show that peripheral venous serum from juvenile diabetics contains immunologically active insulin, and that the activity of this type of insulin is not increased by a rise in the blood sugar. This is in agreement with the observations that there was no increase in immunologically active insulin in spite of considerably increased

active insulin, triggered-off by elevated blood sugar, is less in the older non-obese diabetics. This assumption is compatible with the fact that after glucose administration, the rise in immunologically active serum insulin is slower in these patients than in normal subjects.

Older obese diabetics

When peripheral venous blood is examined from fasting older diabetics, most studies by the rat diaphragm method find elevated SILA levels (see page 69), and in one of two investigations an increased suppressible SILA was found in patients who had not been on a diet (see page 70). Examining older obese diabetics by the rat epididymal fat method, it was found that these patients had elevated fasting SILA values when on normal diet, but that SILA and fasting blood sugar fell when they were on a diet with restricted carbohydrate content (see page 69). Correspondingly studies by the rat diaphragm method showed normal fasting SILA values in older obese diabetics on a diet (see page 69). These findings seem to indicate that untreated patients with this type of diabetes mellitus have elevated immunologically active insulin in the peripheral venous blood, and that the level becomes normalized during treatment with restricted carbohydrate intake.

Some time after ingestion of glucose, a rise in SILA is found (rat diaphragm method) in peripheral venous blood in older obese diabetics (see page 69), just

as there is a rise in suppressible SILA (see page 70). Shortly after intravenous administration of glucose, however, no change is found in suppressible SILA (see page 70). These findings are in agreement with the assumption that after glucose administration, the amount of immunologically active insulin in peripheral venous blood increases in older obese diabetics, but that this rise occurs more slowly in these patients than in normal subjects.

Studies on the occurrence of immunologically inactive insulin in peripheral venous blood from older obese diabetics all appear to indicate the presence of an increased concentration of this type of insulin in such patients, presumably localized to the B fraction (see page 74). It is not clear whether a fall in the immunologically inactive insulin occurs in this type of diabetic during dietary treatment. Thus, when serum from obese diabetics under dietary treatment was extracted by means of Baird & Bornstein's method, elevated values for immunologically determined insulin were found (see page 72), but when serum from such patients was examined by the rat epididymal fat method, no increase in SILA was observed on dilution of the serum (78). If the "dilution effect" depends on an activation of immunologically inactive insulin (see page 51) this last observation will be compatible with a decreased level for this type of insulin, whereas the first investigation reported suggests the opposite.

On the basis of studies on serum insulin in peripheral venous blood from

for the insulin antagonism demonstrated in serum from such patients, is unknown (273) The serum insulin antagonism could be just as well primary as secondary, i.e. a result of the abnormal insulin metabolism. It is also possible that both abnormalities are caused by the same diabetogenic factor in juvenile patients with diabetes mellitus.

Older, non-obese diabetics

In the great majority of studies on peripheral venous blood from non-obese older diabetics, normal or low normal fasting values were found for immunologically-determined insulin, suppressible SILA (see page 70), and SILA determined by the rat diaphragm method (see page 69). As the patients examined showed an elevated fasting blood sugar, these studies suggest that older non-obese diabetics have a relatively reduced level of immunologically active insulin in peripheral venous blood. Using the rat diaphragm method, a rise in SILA was found in these patients after oral glucose (see page 69), presumably indicating an increase in immunologically active insulin. Admittedly, this does not fit in with the finding of unchanged suppressible SILA in older non-obese diabetics after ingestion of glucose (see page 70). However, considering that this result was obtained in a study including only 5 patients, whereas the investigation of SILA by the rat diaphragm method comprised a considerably more extensive patient material, the result of the latter study must be re-

garded as the more likely. In agreement with this assumption, several of the investigations using the rat epididymal fat method have demonstrated a rise in SILA some time after administration of glucose in older non-obese diabetics (see page 69), since this rise may well be due to immunologically active insulin. When suppressible SILA was determined shortly after intravenous administration of glucose, however, unchanged values were found (see page 70).

Investigations of the changes in immunologically active insulin after glucose administration in older non-obese diabetics, agree with the assumption that the activity of this type of insulin increases after glucose administration, but that the rise occurs more slowly than in normal subjects. In agreement with this, immunologically-determined insulin showed a slower rise in a great number of older, untreated diabetics (whose further data were not provided), than in normal subjects (see page 70).

The studies which have so far aimed at elucidating the characteristics of serum insulin in older non-obese diabetics, appear to be unanimous in showing an insulin production in these patients. In peripheral venous blood, however, relatively reduced fasting values for immunologically active insulin seem to be found together with normal or low normal values for immunologically inactive insulin, it appears likely, therefore, that the insulin metabolism in these patients is similar to that of normal subjects but that the production of immunologically

41

Eskjær Jensen et al
(1963) ^a

60)	700(12) normal
60 ¹)	120 b) normal
120 ¹	315(6) decreased
60 ¹)	540(7) low normal
60 ¹)	380(5) decreased
60 ¹)	360(12) decreased
60 ¹)	424(7) decreased

—	Phear (1962) ¹⁰¹
—	
—	
—	Dawcke (1963 ¹⁰² 1963) ¹⁰²
—	
+	
+	

older obese diabetics, it must be concluded that an insulin production is present in these patients, and since the level of immunologically active insulin varies with changes in the blood sugar, it seems reasonable to assume that the insulin production responds to changes in the blood sugar. In dietary-treated diabetics of this type, however, when normal SILA values (presumably reflecting a normal level of immunologically active insulin) are found together with elevated fasting blood sugar, this may be taken as expressing an "abnormality of regulation" in the insulin production. Our knowledge of insulin metabolism in normal subjects is too inadequate, however, to decide whether this hypothesis is compatible with the findings of a higher level of immunologically inactive insulin in the B fraction, and, following glucose administration, of a slower rise in immunologically active insulin in peripheral venous blood, than in normal subjects.

The hypothesis presented, however, does not explain why increased fasting blood sugar is found in untreated older

obese diabetics, in spite of an elevated fasting level of immunologically active insulin and a presumably increased production of insulin. Nevertheless, investigations suggest that these patients have an increased production of growth hormone before they commence treatment (75, 78), and this may give rise to a decreased insulin sensitivity in the musculature (88). It is reasonable, therefore, to assume that an increased production of growth hormone may cause an increase in fasting blood sugar, in spite of a presumably increased production of insulin. Whether the cause of the diabetic condition in the older obese patients is an increased production of growth hormone, or whether this increase is secondary to the diabetes, is nevertheless not clear. This problem might be solved by studies on the production of growth hormone and insulin in the prediabetic condition in older obese patients, as well as on the changes in the amount of the different types of insulin in peripheral venous serum from older obese diabetics under treatment with diet and growth hormone.

INSULIN CONTENT OF BLOOD OF PATIENTS OBESITY AND PATIENTS WITH ACROMEGALY

inulin in these patients
cellulitis has been investi-
that number of studies see-
are pending, investigations
the patients are few
Table 18 shows that normal

or high normal fasting SIIA values are found in peripheral venous blood from such patients (the rat epididymal fat method) as well as high normal fasting values for immunologically determined insulin after extraction of the serum by acid alcohol. In one study, 223 different levels were found for immunologically determined insulin in

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h	B	al	at 200 4 %	normal		
in		gl u al	800 10	high norm 11	Phear (1962) 11	
	d	al			Lingwe (1962) 114	
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CHAPTER 6

THE INSULIN CONTENT OF BLOOD OF PATIENTS WITH OBESITY AND PATIENTS WITH ACROMEGALY

SERUM INSULIN IN NON-DIABETIC PATIENTS WITH OBESITY

While serum insulin in obese patients with diabetes mellitus has been investigated in a great number of studies (see page 79) corresponding investigations on non-diabetic obese patients are few in number. Table 18 shows that normal

or high normal fasting SIIA values are found in peripheral venous blood from such patients (the rat epididymal fat method) as well as high normal fasting values for immunologically determined insulin after extraction of the serum by acid alcohol. In one study (273) different levels were found for immunologically determined insulin in

TABLE 18
Serum insulin in non-diabetic patients with obesity

Method	Insulin fasting μ U/ml	Insulin 60 min after drinking glucose	
Van der Haeghe & Saks	34,000-6 9,200-30	increased normal	150,000-6 50,200-40
Rat epididymal fat method	62-10 8-7	high normal normal	800-10 high normal
Yalow & Bertram's method	31 f	increased	
Immunological method	60-10	high normal	140-10
		increased	

(non-diabetic patients with obesity)
 Androide patients with obesity
 fasting plasma
 after intravenous administration of glucose
 The figures in brackets indicate the number of sera in each group

tients with and without diabetic metabolic anomaly

These investigations on SILA in patients with acromegaly make it reasonable to assume that an increased production of growth hormone may cause an increase in SILA. This assumption is in agreement with studies in which an elevated fasting SILA was found after administration of growth hormone to normal subjects (Zahnd et al 306) and to experimental animals (205). Stein & co-workers (251), however, have not been able to confirm these findings by administration of growth hormone to normal subjects although these investigators used a technique which is almost identical to that of Zahnd with the exception that the growth hormone was administered over a longer period.

It is of considerable interest that Zahnd & co-workers (306) found a rise in SILA 4 hours after intravenous administration of growth hormone without the occurrence of a corresponding fall in the blood sugar. Assuming that the rise in SILA reflects an increased

production of insulin, a fall in the blood sugar might have been anticipated concurrently with the rise in SILA. As this does not seem to be the case, it is reasonable to assume that the growth hormone has caused a decreased sensitivity to insulin in the musculature (88), and to consider the increased insulin production as being a compensatory reaction to this. An interesting observation in this connection is that after long term administration of growth hormone to experimental subjects, Stein & co-workers (251) found that glucose ingestion was followed by a much higher rise in SILA level in these subjects than in normal subjects who had not been treated with growth hormone. The assumption that an increased production of insulin following administration of growth hormone expresses an indirect effect, gains support from a recently published study (104). This shows that isolated perfused rat pancreas does not increase its production of immunologically-determined insulin after stimulation with growth hormone.

serum extracts from obese patients of two differing types, elevated fasting values in "gynoid" and normal fasting values in "android" obese patients. The investigations mentioned deserve attention in spite of the uncertainty of the objective basis for this distribution of obesity into two types, and in spite of the limited number of patients studied.

In peripheral venous blood from obese patients, glucose administration was followed by a rise in SILA and in immunologically-determined insulin in serum extracts, to values higher than those determined in serum from normal subjects (table 18).

A single investigation of immunologically-determined insulin in arterial blood from obese patients showed elevated fasting values (table 18).

The investigations cited, in which serum insulin is studied in non diabetic patients with obesity, do not permit any precise description of the abnormalities which are present in the insulin metabolism of these patients. In view of the fact that both elevated SILA and immunologically-determined insulin may be found after glucose administration, it nevertheless seems reasonable to assume that non-diabetic patients with obesity will respond to glucose administration by an increased production of insulin. It is probable that the increased fasting level of immunologically-determined insulin in peripheral arterial blood is due to the fact that the insulin production is also increased during the fasting state. There are no investigations, however, elucidating the type of stimuli

which set off the increased insulin production, just as it is not known whether the increased insulin production is secondary or primary to the development of obesity.

SERUM INSULIN IN PATIENTS WITH ACROMEGALY

The most comprehensive investigations elucidating serum insulin in acromegalic patients have been made with the rat diaphragm method. Randle (200, 204) found definitely elevated SILA values in patients with acromegaly, while Wright (304) found increased fasting SILA in acromegalic men, but not in acromegalic women.

Examination of 3 acromegalic patients by means of the rat epididymal fat method showed high normal values (155), whereas Yalow & Berson (308) found a normal fasting level of immunologically-determined insulin in two patients. Investigations of immunologically-determined insulin in acid alcohol extracts from serum showed elevated values in 3 acromegalic patients (130). Wright (304) compared the SILA level in acromegalic patients with varying "activity" of the disease, but found no relationship. This seems a reasonable result in view of the difficulties of deciding whether a patient is suffering from active or inactive acromegaly. On the other hand, it is astonishing that none of the more comprehensive investigations on acromegaly have made any comparisons between SILA levels in pa-

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Examination of 3 acromegalic patients by means of the rat epididymal fat method showed high normal values (155), whereas Yalow & Berson (308) found a normal fasting level of immunologically-determined insulin in two patients. Investigations of immunologically-determined insulin in acid alcohol extracts from serum showed elevated values in 3 acromegalic patients (130). Wright (304) compared the SILA level in acromegalic patients with varying "activity" of the disease, but found no relationship. This seems a reasonable result in view of the difficulties of deciding whether a patient is suffering from active or inactive acromegaly. On the other hand, it is astonishing that none of the more comprehensive investigations on acromegaly have made any comparisons between SILA levels in pa-

mellitus exhibit different abnormalities of serum insulin. The factors responsible for these abnormalities are not known, and at present no more can be concluded than that all diabetics must be assumed to have an abnormal insulin production and/or metabolism. Whether these abnormalities in themselves cause diabetes mellitus, or whether they are secondary to other conditions which determine the diabetic state, is not known. It is an obvious point in this connection that an abnormal production of serum insulin antagonists in the prediabetic and diabetic states may be assumed to involve abnormalities in the serum insulin. Our present knowledge, however, does not permit us to decide which of the antagonists described is responsible and it certainly does not

permit us to formulate any conclusions as to how the mechanism functions. It should also be stressed that the whole question of the type and number of serum insulin antagonists is so unsettled that it is only with the greatest caution that any importance dare be ascribed to them in the pathogenesis of diabetes. One of the most significant tasks for the future, therefore, must be to elucidate the relationship between the serum insulin antagonists so far described. With this achieved, it will be reasonable to suppose that studies on the occurrence of serum insulin antagonism, on the different types of serum insulin in "prediabetics" and on different types of experimental diabetes, will increase our knowledge of the pathogenesis of diabetes mellitus.

FINAL COMMENTS

Our knowledge with regard to serum insulin has been considerably extended during recent years, but at the same time our conception of the properties of the insulin in serum has become more complex. It must now be accepted as a fact that untreated serum contains considerable amounts of insulin in a form which is biologically inactive *in vitro*, so that more than one type of insulin is present in serum. As a result of recent studies showing that commercially purified pancreatic insulin may be antigenic to the animal species from which it has been prepared, the question has been raised whether the insulin molecule in serum differs from the insulin molecule in the pancreas. It is not clear whether this is in some way a consequence of the same phenomenon which is responsible for serum insulin occurring in a variety of forms, or whether it is due to the fact that pancreatic insulin is altered during the extraction procedure. Studies to determine whether the serum insulin is associated with the plasma proteins, and if so, how, may possibly provide a reply to this question, and at the same time may explain what happens when serum is treated by "warm dialysis". Closely related to these problems is the question of the relationship

between the values obtained by the different methods for determining immunologically inactive insulin. It is possible that the terms "non suppressible SILA", "complex insulin" and "ILA of A and B fraction", all represent different insulin fractions, but it is also conceivable that one and the same fraction can be determined by a variety of methods.

As has been discussed previously, our knowledge of the physiological significance of the different types of insulin is very limited. It is probable that immunologically active insulin is biologically active *in vivo*, but whether immunologically inactive insulin has an *in vivo* effect, perhaps only in certain tissues, and whether there is a difference in this respect between the A and the B fractions, is not known. Closely related to this problem are the questions of the site of formation of the different fractions of immunologically inactive insulin, of where they are consumed, and of whether the production and the consumption vary with the metabolic state of the subject.

The present investigations on serum insulin in diabetics have furnished us with the very significant information that different clinical types of diabetes

described. The mean concentration of immunologically active insulin in normal fasting serum amounts to between 10 and 50 μ U/ml, the concentration increasing following administration of glucose. This type of insulin is produced in the pancreas and is partly retained in the liver, and presumably also peripherally. The normal values for immunologically inactive insulin vary considerably, depending on the methods used to determine this type of insulin. The sites of formation and of consumption of immunologically inactive insulin are not known.

Chapter 5

Studies on the serum insulin in patients with diabetes mellitus have shown that insulin is present in the serum in all types of diabetes, but that it differs in a number of ways from the insulin in normal subjects. Different clinical types of diabetes show different kinds of serum insulin abnormality. Relatively low values of immunologically active insulin are found in peripheral venous serum from fasting juvenile diabetics. No rise is observed in this type of insulin following administration of glucose. Juvenile diabetics have presumably an increased amount of immunologically inactive insulin in serum, corresponding to both the A fraction and the B fraction.

Peripheral venous serum from older non-obese diabetics shows relatively low fasting values for immunologically active insulin. The rise in concentration of this type of insulin after glucose administration is slower in these patients than in normal subjects. These diabetics show normal amounts of immunologically inactive insulin, presumably corresponding to both the A and the B fraction.

Elevated values of immunologically active insulin are found in peripheral venous blood from untreated, fasting, older obese diabetics. After glucose administration the rise in this insulin is slower in these patients than in normal subjects. This type of diabetic shows an increased concentration of immunologically inactive insulin, presumably localized to the B fraction.

Chapter 6

Studies on patients with obesity and patients with acromegaly indicate that these two diseases are accompanied by an increased insulin production. The mechanisms triggering off this production in patients with obesity are unknown. Growth hormone, however, is known to be insulinotropic, but whether this is a direct or an indirect effect is uncertain.

SUMMARY

Chapter 1

The methods for determining insulin in small concentrations are discussed, with a view to their applicability to determining insulin in serum. None of these methods are specific for the biologically active insulin molecule. In normal serum, however, the rat diaphragm method, the rat epididymal fat method and the immunological methods based on Yalow & Berson's principle will presumably determine insulin alone.

Chapter 2

Studies are discussed which throw light on the occurrence of different types of serum insulin. With this background, the theory is presented that two types of insulin exist in serum. One type—the immunologically active type—can be bound to anti insulin, while the second type—the immunologically inactive type—cannot be so bound. The immunologically inactive type of insulin presumably occurs in at least two fractions. One fraction is localized to albumin α_2 -globulin, the other fraction to β_1 -globulin.

Chapter 3

Serum insulin antagonists have been demonstrated in normal subjects, diabetics, and animals with experimental diabetes. All the antagonists described can be demonstrated by the rat diaphragm method. For most of the antagonists, it is not reported whether they are active in relation to rat epididymal fat, but the synalbumin antagonist described by Vallance Owen cannot be demonstrated by this method. On the other hand, the method determines antagonism in serum from patients in diabetic acidosis. Several of the antagonists described in man and experimental animals are dependent on an intact hypophyseal and adrenocortical function, but it is not known whether any of the antagonists described in experimental animals correspond to those known in man. Nor is it clear whether some of the insulin antagonists described in man are in fact identical with each other.

Chapter 4

The studies on serum insulin in normal subjects and experimental animals are

described. The mean concentration of immunologically active insulin in normal fasting serum amounts to between 10 and 50 $\mu\text{U/ml}$, the concentration increasing following administration of glucose. This type of insulin is produced in the pancreas and is partly retained in the liver, and presumably also peripherally. The normal values for immunologically inactive insulin vary considerably, depending on the methods used to determine this type of insulin. The sites of formation and of consumption of immunologically inactive insulin are not known.

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Peripheral venous serum from older non obese diabetics shows relatively low fasting values for immunologically active insulin. The rise in concentration of this type of insulin after glucose administration is slower in these patients than in normal subjects. These diabetics show normal amounts of immunologically inactive insulin, presumably corresponding to both the A and the B fraction.

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RESUME

Kapitel 1

Methoderne til registrering af insulin i små concentrationer gennemgås med henblik på deres anvendelighed til bestemmelse af insulin i serum. Ingen af disse metoder er specifikke for det biologisk aktive insulinmolekyle, men i normalt serum må rottediafragmamethoden, rottepididymusfedtmethode og de immunologiske metoder baseret på Yalow & Bersons princip formodes kun at bestemme insulin.

Kapitel 2

Undersøgelser der belyser forekomsten af forskellige former for seruminsulin diskuteres. På baggrund heraf fremsættes den teori, at insulin forekommer i to former i serum, i den ene form – den immunologisk aktive – kan det bindes til antiinsulin, i den anden form – den immunologisk inaktive – kan det ikke bindes. Den immunologisk inaktive form for insulin må antages at forekomme i mindst to fraktioner, den ene lokaliseret til albumin α_2 -globulin den anden til β - γ -globulin.

Kapitel 3

Seruminsulin antagonist er påvist hos normalpersoner, diabetikere og experi-

mentelt diabetiske forsøgsdyr. Samtlige beskrevne antagonist er påviselige med rottediafragmametoden. For de fleste antagonisters vedkommende er det uoplyst, om de er aktive overfor rottepididymusfedt, men den af Vallance Owen beskrevne synalbuminantagonist kan ikke påvises med denne metode, der derimod registrerer antagonisme i serum fra patienter i diabetisk acidose. Flere af de antagonist, der er beskrevet hos mennesker og forsøgsdyr, er afhængige af intakt hypofyse og binyrebarkfunktion. Men om nogle af de antagonist, der er beskrevet hos forsøgsdyr svarer til de, der kendes hos mennesker er ukendt, og det er ligeledes uklart om nogle af de hos mennesker beskrevne insulinantagonister i virkeligheden er identiske.

Kapitel 4

Undersøgelser af seruminsulin hos normalpersoner og forsøgsdyr omtales. I normalt fasteserum er den gennemsnitlige koncentration af immunologisk aktivt insulin mellem 10 og 50 μ U/ml efter glukose stiger koncentrationen. Den ene form for insulin produceres i pancreas og retineres partielt i leveren og formentlig også i periferen. Normalværdierne for immunologisk aktivt insulin

varierer betydeligt, afhængigt af hvilke metoder der anvendes til registrering af denne form for insulin. Det er ukendt, hvor det immunologisk aktive insulin dannes og forbruges.

Kapitel 5

Arbejder der belyser seruminsulinnets forhold hos patienter med diabetes mellitus har vist, at der hos alle typer diabetikere findes insulin i serum, men at dette på flere måder forholder sig anderledes end hos normalpersoner. Seruminsulinabnormiteterne er af forskellig art hos diabetikere af forskellig klinisk type. I perifert venøst serum fra fastende juvenile diabetikere findes relativt nedsatte værdier for immunologisk aktivt insulin, efter glukoseindgift ses ingen stigning i denne form for insulin. De juvenile diabetikere har antagelig en øget mængde immunologisk inaktivt insulin i serum såvel svarende til A som til B fraktionen. I perifert venøst serum fra ældre ikke adipøse diabetikere findes relativt nedsatte fasteværdier for immunologisk ak-

tivt insulin. Efter glukoseindgift stiger koncentrationen af denne form for insulin langsommere end hos normalpersoner. Hos disse diabetikere findes normale mængder immunologisk inaktivt insulin formodentlig såvel svarende til A som til B fraktionen. I perifert venøblod fra ubehandlede, fastende, ældre adipøse diabetikere findes forhøjede værdier for immunologisk aktivt insulin, efter glukoseindgift stiger dette langsommere end hos normalpersoner. Hos denne type diabetikere findes en øget koncentration af immunologisk inaktivt insulin, antagelig lokaliseret til B fraktionen.

Kapitel 6

Undersøgelser af patienter med adipositas og patienter med acromegali tyder på, at disse to sygdomme er ledsaget af en øget insulinproduktion. Det er ukendt, hvilke mekanismer, der udløser denne hos patienter med adipositas. Derimod vides det, at væksthormon er insulinotrop, men om dette er en direkte eller indirekte effekt er uvist.

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UNIVERSITY OF LUND

HEAD PROFESSOR IAN WALDENSTRAEM

CLINICAL STUDIES ON KIDNEY FUNCTION
WITH RADIOACTIVE SODIUM DIATRIZOATE
(HYPAQUE®)

BY

TORSTEN DENNEBERG

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INTRODUCTION

Hypaque® (sodium 3,5 diacetamido 2,4,6 triiodobenzoate or sodium diatrizoate) has been widely used in the last decade in excretion urography. Labelled with ^{131}I the substance (^{131}I Hypaque) has also been used in clearance studies and external renal measurements (radioisotope renogram). Despite this extensive use the relative roles of glomerular and tubular function in the excretion of ^{131}I Hypaque

are still obscure. Better knowledge of the disappearance of the substance from the plasma, its renal and extra-renal excretion as well as its clearance would be useful in the evaluation of the possibility of using ^{131}I Hypaque as a substitute for inulin in the measurement of glomerular filtration and as a suitable substance for single injection clearance tests and in the interpretation of renograms.

rather low incidence of untoward reactions and the very good visualization it offers (Lowman et al. 1955 Moore & Mayer 1955 Harrow 1956 and others). So far, only few cases have been reported in which side effects could be ascribed to the Hypaque (Berlyne & Berlyne 1962 Hansson & Lindholm 1963).

While the excretory mechanism of the previously mentioned contrast media such as Diodrast and Hippuran is well known opinions differ about the mode of excretion of triiodinated benzoic compounds. The first reports on the renal excretion of Hypaque and Urografin were based on animal experiments. Langecker et al. (1954) who used dogs showed that at plasma concentrations about or below 1 mg/100 ml the Urografin/inulin clearance quotient is more than 1.0, indicating that Urografin is also excreted by the tubules. They concluded that in a dose of 10 g (i.e. amounts used for excretion urography) Urografin is probably excreted mainly by glomerular filtration but when the plasma level is low also by the tubules.

Knoefel & Huang (1956) and Knoefel et al. (1961) studied several triiodinated derivatives and found that substances with an *o*-ethylamido group in the 3 position or in 3 and 5 positions were excreted by the tubules. Hypaque was one of these substances and was found to have a certain maximal rate of tubular excretion.

McCluskey & Hoppe (1957) who used doses of Hypaque in amounts employed in roentgen examinations, found that its clearance was independ-

ent of plasma concentrations between 90—1200 mg/100 ml and that the clearance with doses of this size closely resembled that of inulin. They also noted that Hypaque was apparently not influenced by the simultaneous transport of PAH through the tubules. These findings were interpreted as indicating that in the doses used Hypaque was excreted mainly by glomerular filtration. These animal experiments with stable Hypaque seemed to show that in small doses it is eliminated by tubular excretion as well as by glomerular filtration, but in the large doses used for roentgenography it is excreted mainly by glomerular filtration.

The introduction of radioactive isotope techniques improved the possibilities of studying renal function (Oeser & Bilton 1952 Wossidlo 1952). Oeser & Bilton (1952) used ^{131}I labelled Uroselectan in their investigations of renal function and measured its excretion in the urine by determining the beta ray activity after a single intravenous injection. Bilton & Schlungbaum (1955) elaborated the technique and found that ^{131}I labelled Perabrodil® (iodo-pyrazet) could be used also in clearance studies for estimating renal function and renal plasma flow with the constant intravenous infusion technique. The gamma radiation was utilized for external measurements over the kidneys by Kumbel (1956) who compared ^{131}I Urografin and ^{131}I Perabrodil. The method of external measurement over the kidneys was introduced and systematically developed as a clinical procedure (radioisotope renogram) by Taplin et al. (1956).

SURVEY OF LITERATURE

A Iodinated contrast media used in kidney studies

Several iodinated organic contrast media have proved useful in the investigation of renal function and renal blood flow. The first iodinated organic substance used for excretion urography was that described by Swick (1929), viz Uroselectan® (sodium iodomethamate). Swick (1933) also introduced Hippuran® (sodium o-iodo-hippurate) as a contrast medium for excretion urography. Several other contrast media were studied and tried but iodopyracet, Diodrast® (3,5 diiodo-4 pyridone N acetic acid diethanolamine) soon proved the most suitable and was the medium most frequently used during the following 20 years, it was also widely used in renal clearance studies (Elson et al 1936, Smith et al 1938 and others). Para amino hippuric acid (PAH), whose clearance was identical with that of Diodrast and Hippuran, could be determined chemically in a simple way, which was one of the chief reasons why this substance was most widely used (Smith et al 1945).

In the beginning of the 1950s several iodinated acylaminobenzoic compounds with a still higher iodine con-

tent were studied by Wallingford et al (1952), who found sodium acetrizoate Urokon® (sodium 2 acetamido 2,4,6 triiodobenzoate) to be a suitable urographic agent. A chemically closely related substance is sodium diatrizoate, Hypaque (sodium 3,5 diacetamido 2,4,6 triiodobenzoate) which was synthesized by Larsen et al (1954) in U.S.A. This latter compound was described independently at the same time in Germany by Langecker et al (1954), who carried out a clinical trial with a mixture of the sodium- and N methyl glucamine salts (Urografin® in Europe, Renografin® in U.S.A.).

The high degree of radiopacity of these substances, their low systemic and local tissue toxicity, prompt complete elimination by the kidneys and insignificant pharmacodynamic side effects led to further improvement of the roentgenological diagnosis of renal diseases (Langecker et al 1954, Hoppe et al 1956, Knoefel & Huring 1956, Hoppe 1959, Lindgren & Törnell 1958, Lindgren 1961 and others).

In most clinical studies in which comparisons have been made of various renal contrast media such as Hypaque, Urokon and Diodrast, Hypaque has proved superior because of the

This rapid elimination of the substance from the blood agreed well with the corresponding rapid elimination by the kidneys. No substantial difference in excretion rate appeared to occur when non-radioactive Hypaque or Urografin was used in a dose of 0.5 g/kg or tracer doses. The rapid excretion by the kidneys may be characterized by the following values in dogs: 67 per cent of the dose was excreted in 2 hours, 88 per cent in 8 hours and 92 per cent in 24 hours (McChesney & Hoppe 1957) which was in good agreement with the 24 hour value of 90 per cent reported by Blaufox et al (1963 d).

Kimbel & Borner (1955) found that after injection of labelled Urografin into rats the concentration in all parenchymatous organs and in the blood fell rapidly with the exception of the small and large intestines where about 10 per cent of the dose was still present after 3 hours. After 24 hours it was no longer possible to demonstrate any activity in the animals except in the intestines. McChesney & Hoppe (1957) made similar findings in investigations with non-radioactive Hypaque in cats. The pattern of the tissue distribution suggested that most of the Hypaque was located in the extracellular fluid and with time Hypaque was rather rapidly removed and transported to the kidneys for excretion. No special predilection for any other organ or tissue was observed.

Impecker et al (1954) found 0.5 and 0.7 per cent of the dose of non-radioactive Urografin in the bile in rabbits after 2 and 6 hours, respec-

tively. These findings agreed with those reported by Hansson & Lindholm (1963) in rabbits and Blaufox et al (1963 d) in dogs while Winkler & DeMaria (1961) and Kimbel (1963) reported somewhat higher values with addition of carrier to the radioactive substance in rats. While the excretion in the bile was insignificant in normal rabbits it was as high as 20 per cent in nephrectomized animals. The values with low biliary excretion agreed well with those reported by McChesney & Hoppe (1957) who found that the faecal excretion in dogs was 0.7 per cent in 24 hours and 2 per cent in 72 hours.

Investigations on animals have thus shown that under normal conditions Hypaque or Urografin is eliminated rapidly from the blood by the kidneys and that no substantial uptake occurs in any other organ and that there is no significant extrarenal excretion. The picture is quite different in experimental anuria when the excretion in the bile and by the intestines increases considerably.

Studies in human beings

Since the blood concentration curves and the disappearance rate of ^{131}I Hypaque as well as its distribution and its renal excretion will be discussed in detail in Chapter VI suffice it here to say that the various investigators have analysed the blood curves with the help of slope analysis (Schlungbaum & Billion 1956 b, Denenberg et al 1961 & Stokes & Ter Pogossian 1964).

These ^{131}I labelled contrast media have been used 1) as substitute for a corresponding non radioactive medium in clearance studies, 2) for external measurement over the kidneys (radio isotope renogram), 3) for renal scanning and scintigraphy and 4) in combinations of the above mentioned examinations for determination of total renal function and of the performance of each kidney separately

Experimental investigations on animals and trials on human beings with the single injection technique for elucidating the distribution and kinetics of the radioactive substances have been reported by several authors. In these experiments the blood concentration curves and corresponding external curves over different parts of the body (head, foot, heart and kidneys) have been analysed. Animal experiments with direct measurements of radioactivity of organs or with autoradiographic technique have also been performed (Kimbel & Börner 1955, Denneberg et al 1960, Magnusson 1960 1962, zum Winkel & DeMaria 1961, Schlungbaum 1962 zum Winkel 1964). The single injection technique has been used to study the mechanism of the renal excretion of the substance (Bianchi 1961 Denneberg et al 1961 a, Bianchi & Toni 1962 Golt et al 1962 Blaufox et al 1963 b d Meschan et al 1963 a, c).

With the aid of the continuous intravenous infusion technique according to the principles described by Homer Smith (1951) labelled and unlabelled substances have been compared regarding their renal excretion. The

purpose was to substitute the non radioactive substance by the radioactive agent for determination of glomerular filtration, tubular secretion and renal blood flow (Billion & Schlungbaum 1955, Kinter & Pappenheimer 1956, Schlungbaum & Billion 1956 a, Bergstrom et al 1959, Block & Burrows 1960, Parker & Beierwaltes 1960, Bianchi & Zampieri 1961, Burbank et al 1961, Schwartz & Madeloff 1962).

The double isotope technique using labelling of substances with either ^3I or ^{131}I opened further possibilities of using these substances in the diagnosis of renal diseases (Stokes & Ter Pogossian 1964). For this purpose several investigations were made with labelled Diodrast and Hippuran for determining renal plasma flow, and various labelled trisodinated substances, particularly Hypaque (Renografin or Urografin), were studied for their value as substitutes for inulin in the measurement of glomerular filtration.

B Distribution and excretion of sodium diatrizoate (Hypaque, Renografin, Urografin)

Experiments in animals

Kimbel & Börner (1955) found that the blood curves obtained after a single injection of radioactive Urografin fell rapidly during the first 3 hours and after 24 hours it was hardly possible to demonstrate any activity in the blood. Similar findings have been reported by Hansson & Lindholm (1963) with radioactive Hypaque in rabbits.

This rapid elimination of the substance from the blood agreed well with the corresponding rapid elimination by the kidneys. No substantial difference in excretion rate appeared to occur when non-radioactive Hypaque or Urografin was used in a dose of 0.5 g/kg or twice doses. The rapid excretion by the kidneys may be characterized by the following values in dogs: 67 per cent of the dose was excreted in 2 hours, 88 per cent in 8 hours and 92 per cent in 24 hours (McChesney & Hoppe, 1957) which was in good agreement with the 24 hour value of 90 per cent reported by Blaufox et al (1963 d).

Kimbel & Borner (1955) found that after injection of labelled Urografin into rats the concentration in all parenchymatous organs and in the blood fell rapidly with the exception of the small and large intestines where about 10 per cent of the dose was still present after 4 hours. After 24 hours it was no longer possible to demonstrate any activity in the animals except in the intestines. McChesney & Hoppe (1957) made similar findings in investigations with non-radioactive Hypaque in cats. The pattern of the tissue distribution suggested that most of the Hypaque was located in the extracellular fluid and with time Hypaque was rather rapidly removed and transported to the kidneys for excretion. No special predilection for any other organ or tissue was observed.

Langbecker et al (1954) found 0.5 and 0.7 per cent of the dose of non-radioactive Urografin in the bile in rabbits after 3 and 6 hours respec-

tively. These findings agreed with those reported by Hansson & Lindholm (1963) in rabbits and Blaufox et al (1963 d) in dogs while Winkler & DeMaria (1961) and Kimbel (1963) reported somewhat higher values with addition of carrier to the radioactive substance in rats. While the excretion in the bile was insignificant in normal rabbits it was as high as 25 per cent in nephrectomized animals. The values with low biliary excretion agreed well with those reported by McChesney & Hoppe (1957) who found that the faecal excretion in dogs was 0.7 per cent in 24 hours and 2 per cent in 72 hours.

Investigations on animals have thus shown that under normal conditions Hypaque or Urografin is eliminated rapidly from the blood by the kidneys and that no substantial uptake occurs in any other organ and that there is no significant extrarenal excretion. The picture is quite different in experimental anuria when the excretion in the bile and by the intestines increases considerably.

Studies in human beings

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These ^{125}I -labelled contrast media have been used 1) as substitute for a corresponding non radioactive medium in clearance studies, 2) for external measurement over the kidneys (radioisotope renogram), 3) for renal scanning and scintigraphy and 4) in combinations of the above mentioned examinations for determination of total renal function and of the performance of each kidney separately

Experimental investigations on animals and trials on human beings with the single injection technique for elucidating the distribution and kinetics of the radioactive substances have been reported by several authors. In these experiments the blood concentration curves and corresponding external curves over different parts of the body (head, foot, heart and kidneys) have been analysed. Animal experiments with direct measurements of radioactivity of organs or with autoradiographic technique have also been performed (Kimbél & Börner 1955, Denneberg et al 1960, Magnusson 1960, 1962, zum Winkel & DeMaria 1961, Schlungbaum 1962, zum Winkel 1964). The single injection technique has been used to study the mechanism of the renal excretion of the substance (Bianchi 1961, Denneberg et al 1961 a, Bianchi & Toni 1962, Gott et al 1962, Blaufox et al 1963 b, d, Meschan et al 1963 a, c).

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Kimbel & Börner (1955) found that after injection of labelled Urografin into rats the concentration in all parenchymatous organs and in the blood fell rapidly with the exception of the small and large intestines where about 10 per cent of the dose was still present after 3 hours. After 24 hours it was no longer possible to demonstrate any activity in the animals except in the intestines. McChesney & Hoppe (1957) made similar findings in investigations with non-radioactive Hypaque in rats. The pattern of the tissue distribution suggested that most of the Hypaque was located in the extracellular fluid and with time Hypaque was rather rapidly removed and transported to the kidneys for excretion. No special predilection for any other organ or tissue was observed.

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the filtered ^{131}I Hypaque was reabsorbed during high urine flow rate. The increase in ^{131}I Hypaque clearance during osmotic diuresis was believed to reflect reabsorption of ^{131}I Hypaque in the proximal tubules. They concluded, however, that ^{131}I Hypaque clearance in dogs is predominantly a function of glomerular filtration.

While the above mentioned authors compared ^{131}I Hypaque with creatinine Meschan et al (1963 a) compared radioactive Renografin without addition of carrier and inulin. They found that the clearance quotient was between 0.92 and 1.15 in 13 separate tests on 5 dogs. The mean of the clearance quotients was 1.01. They therefore concluded that ^{131}I Renografin is a good substitute for inulin in measurement of glomerular filtration. Investigations on dogs have thus given contradictory results but the possibility of a partial tubular secretion and reabsorption can not be excluded. Since no experiments have been performed with inhibitors of tubular activity the problem is not definitely solved.

Studies in human beings

The mechanism of renal excretion of Hypaque in human beings has been studied by a number of investigators using different isotope methods (Branchi 1961, Branchi & Zampieri 1961, Dencher et al 1961 a, Schlunghaus 1962, Branchi & Toni 1963, Tauxe et al 1964, Morris et al 1965). They all found ^{131}I Hypaque to have a lower renal clearance value than substances of the type ^{131}I Diodrast, PAH and ^{131}I

Hippuran but opinions differ as to whether it is excreted by glomerular filtration alone or also partially by tubular secretion. These problems will be discussed in detail in Chapter VII.

D Studies on interaction between sodium diatrizoate and serum proteins

In the investigation of renal function knowledge of the protein binding of the test substance used is of importance. Homer Smith (1951) pointed out that although protein binding may retard diffusion from the peritubular capillaries into the interstitial fluid it will not render the substance unavailable for tubular excretion because the protein complex may be rapidly reversible. Reduction of concentration of the substance in the interstitial fluid on uptake by the tubules is immediately compensated by diffusion of the substance from the capillaries with consequent reduction of its concentration in the plasma. In principle all of the substances contained within the plasma may dissociate and escape by diffusion before the blood emerges from the peritubular capillaries and this does in fact occur. Proof of this is shown by identical clearance rates of Diodrast, Hippuran and PAH though each of these substances exhibits a different degree of protein binding. When calculating the amount of a substance filtered through the glomeruli on the other hand the binding on protein is believed to be of importance.

and all the investigators found ^{131}I -Hypaque or ^{131}I -Urografin to be rapidly eliminated from the blood. Examinations of the excretion rate of ^{131}I -Urografin gave values compatible with the rapid elimination from the blood (Langecker et al 1954, Kimbel & Börner 1955, Schlungbaum & Billion 1956 b). The excretion by extra-renal routes were negligible or insignificant (bile, gastric juice, saliva), the faecal excretion was between 1—2 per cent (Kimbel & Börner 1955, Schlungbaum & Billion 1956 b).

C Renal excretion mechanism of sodium diatrizoate (Hypaque, Renografin, Urografin)

Experiments in animals

The mechanism of the renal excretion of Hypaque has been studied in animals with various isotope methods. The majority of investigators have concluded that Hypaque is excreted mainly by glomerular filtration but as mentioned above findings by Langecker et al (1954) argued for an excretion by the tubules when non radioactive Urografin was given in doses about or below 1 mg/100 ml. In comparison between radioactive Diodrast and Urografin Willenbrink & Kimbel (1959) found that both were excreted by aglomerular marine teleost fishes, but that the excretion of Diodrast was much greater (during 24 hours 16.2 per cent and 3.1 per cent respectively). In rabbits however they found that ^{131}I Urografin clearance was unchang-

ed at plasma concentrations of 1—1000 mg/100 ml and that saturation of the tubules with PAH did not influence the rate of excretion of ^{131}I Urografin. They therefore thought that in this range of plasma concentrations ^{131}I Urografin was excreted only by glomerular filtration.

In cats Denneberg et al (1960) studied the tissue distribution and renal excretion of radioactive Hypaque, Diodrast and Urokon with auto radiography and external measurements. They found that the three substances accumulated in the renal cortex and in the medullary zone and were rapidly eliminated into the pelvis. All the substances accumulated in the tubules, but they differed in the rate of excretion to the renal pelvis. They concluded that ^{131}I Hypaque was probably excreted by the same mechanism as ^{131}I Diodrast in the cat.

Woodruff & Malvin (1960), who used stop flow analysis and the infusion clearance technique with ^{131}I Hypaque in dogs, found the clearance to be independent of the plasma level. Judging from the clearance data a small amount of the filtered mass appeared to be reabsorbed. However stop flow and protein binding studies revealed ^{131}I Hypaque to be handled exactly like creatinine by the kidney. Stokes et al (1962) found no significant change in the quotient ^{131}I Hypaque/creatinine with plasma concentrations between 0.02 and 94 mg/100 ml. The quotient varied between 0.52 and 0.89 at the above plasma levels but increased up to 0.95 during osmotic diuresis. Approximately 20 per cent of

reported their first experiences with the method in animal experiments and in human beings with various renal diseases. Winter (1956-1957) was the first to compare the isotope renogram with roentgenographic findings and the results of conventional renal function tests in a clinical series. He described the test as a screening test for demonstrating unilateral renal injury in patients with hypertension.

The now voluminous literature on radioisotope renograms in the investigation of various renal diseases has been reviewed by Magnusson (1962), Winter (1963) and zum Winkel (1964). These publications survey the experimental and clinical development of the technique. One of the most important factors in the development of the renogram test was the choice of a suitable test substance. Various labeled iodinated substances have been tried such as Diodrast, PAH, Hippuran as well as substances of the type triiodobenzate (Hypaque, Urografin, Renografin, Mikon and Urokon).

Taplin et al. (1956) found ^{131}I Urokon to be useful in animal experiments but not to be suitable for clinical use. According to these authors ^{131}I Diodrast with its high renal clearance appeared to be the most suitable substance. But this substance, like diiodo-PAH, ^{131}I is excreted not only by the kidneys, but also partly by the liver and the biliary ducts. The extent of this excretion is such that the radioactivity in the liver affects the external renal measurements on the right side and thus interferes with the com-

parison between the two sides (Winter & Taplin 1958, Denneberg & Hedenstam 1959a, Dollery 1960, Bianchi 1961).

In trials with ^{131}I Hypaque the excretion by the liver and biliary ducts did not prove disturbing but the ascent of the second segment and the fall of the third segment were more gentle than in curves obtained with ^{131}I Diodrast (Winter & Taplin 1958). ^{131}I -Hippuran which was introduced by Nordyke et al. (1960) proved to be comparable to ^{131}I Diodrast regarding its uptake by the kidneys but without the undesired excretion by the hepatic and biliary ducts. This substance has therefore been used most widely in recent years for renography.

While the choice of the test substance may be regarded as decided, some factors in the technique described by Taplin et al. (1956) have been modified by other investigators in order to improve the accuracy of the method and to facilitate the measuring procedure. Factors of importance that have received attention in these modifications are the position of the patient during the measurements (sitting, prone or supine), the localisation of the kidneys for exact adjustment of the detectors and collimation of the crystal and detector. In addition to these technical variables simultaneous external measurements over the urinary bladder (renocystogram) or selected parts of the body (heart, head, foot) have contributed to our knowledge of the elimination of the substance from the blood and its arrival in the blad-

According to Homer Smith (1951), the most suitable methods for studying plasma protein binding of renal test agents is ultrafiltration and dialysis through membranes impermeable to plasma proteins. It is, however, remarkable that investigators have obtained widely discrepant results (Smith & Smith 1938, Block & Burrows 1960, Magnusson 1962 and others). This also holds for Hypaque and Urografin. Lasser et al. (1962) underlined the large difference in this respect between species, which may explain the discrepancies between the results obtained by various investigators, but it also appeared probable that the cause of the differences should be sought in differences between the methods used. Thus Langecker et al. (1954), who used ^{131}I Urografin *in vitro*, found with the technique of ultrafiltration at plasma levels from 6.5 to 42 mg per cent values varying from 24.8 to 31.8 and 26.3 to 36.0, respectively, in the percentage bound to the plasma proteins in dogs. Also Knoefel & Huring (1956) used ultrafiltration in experiments in dogs and found that 11–24 per cent of Hypaque was bound to serum proteins. On ultrafiltration with semipermeable membranes or equilibrium dialysis other investigators such as Woodruff & Malvin (1961), Lasser et al. (1962) and Stokes et al. (1962), found that ^{131}I Hypaque was only slightly bound to the serum proteins (5–10 per cent) in dogs and in several other species including human beings (Lasser et al. 1962 and Kimbel 1963).

E Studies on radioisotope renograms

The external renal measuring technique with gamma sensitive scintillation detectors after administration of tracer doses of ^{131}I labelled substances made it possible to determine the isotope at a distance (Taplin et al. 1956). The novelty of this technique was that it provided a possibility to appraise the function of each kidney separately and to demonstrate any disparity between them. The time radioactivity curves obtained with such a technique consist of the following 3 segments: (a) an initial short upswing immediately after the injection of the isotope and considered to be a measure of the initial passage of the radioactivity through the vascular bed of the kidney and the surrounding tissues (so called vascular segment); (b) a second segment with a less marked ascent towards a maximum value of the curve. This segment was interpreted as representing the rate of active tubular cell uptake and passage of the substance through the tubuli (so called uptake segment); (c) a third segment following the maximum value was characterized by a precipitous fall in radioactivity (so called excretion segment). This segment was considered to express the urinary excretion and disappearance of the radioactivity from the area seen by the scintillation detector.

Taplin et al. (1956) reported that the test was mainly a qualitative test for the evaluation of differences in function between the kidneys. They

PURPOSE AND PLAN OF THE INVESTIGATION

The purpose of the present investigation was to elucidate the distribution, renal and extra renal excretion of radioactive Hypaque, and the mechanism of the renal excretion of the substance its suitability for external measurement of the radioactivity over the chest and kidneys and to compare the clearance after administration of the substance by intravenous infusion with that noted after a single injection. The purpose of this comparison was to find the most suitable clearance with the single injection technique as a measure of total renal function which might supplement external renal measurements with evaluation of the function of each kidney separately.

Though it is generally agreed that ^{131}I Hypaque is eliminated mainly by glomerular filtration it is not certain whether tubular secretion and reabsorption of the substance occurs in human beings. This is an important question especially concerning the possibility of using ^{131}I Hypaque instead of inulin as a measure of glomerular filtration. One of the purposes of the present investigation was therefore to clear up the question whether tubular secretion plays any role in the clearance of ^{131}I Hypaque. In the elucidation of this problem clearance was studied after simultaneous administration of inulin PAH and ^{131}I

Hypaque with and without blocking of the tubules by Probenecid.

The introduction of radioisotope renography made it possible to study the function of each kidney separately. In the estimation of the clinical value of renography two problems presented themselves, viz the choice of radioactive substance and quantitative evaluation of the external renal curves.

The purposes of this part of the investigation were 1) to find a suitable method for evaluating the renogram 2) to analyse renographic differences between the two kidneys, 3) to analyse the renogram in different types of renal disease 4) to demonstrate any correlation between parameters calculated from the renogram and the external chest curve 5) to demonstrate any correlation between parameters calculated from the renogram and other renal function data with radioactive Hypaque (clearance with intravenous infusion technique renal excretion rate disappearance from blood curve) and other renal function tests (creatinine clearance serum creatinine \times P \times V and maximal specific gravity) 6) to estimate the agreement between the renogram and the findings made at excretion urography laparotomy and post mortem examination 7) to assess the clinical value of renography.

der (Frohlich et al 1959, Bodfish & Roberti 1960, Scheer & zum Winkel 1960, Säterborg 1960, Abt & Balkus 1961, Feine & Bauer 1961, Johnson et al 1961, Magnusson 1962 and others)

Though the substance is still given as a single injection, other methods using intravenous infusion have also been used (Lindell 1963). In experimental and clinical trials large doses of unlabelled PAH and Probenecid have been given before the isotope test to improve the diagnostic value of the radioisotope renogram (zum Winkel et al 1961 c, Wax & McDonald 1962).

The range of indications for renography has been widened considerably. Radioisotope renography is gentle, it requires only little time, the dose of radioactivity is small and there are no contraindications (Winter 1963). While unilateral renal injury in hypertension has been the commonest indication for examination, the renogram has proved useful also in the investigation of urinary obstruction, in the differential diagnosis between

renal and post renal anuria or oliguria and in the study of the course of functional disorders and the renal excretion after operations on renal arteries, pelvis and ureters. It has also recently proved useful in the postoperative evaluation of renal transplants in man (Collins et al 1963, Loken et al 1964).

Most investigators have found the renogram valuable as a supplement to morphological and other functional methods (excretion urography, retrograde pyelography, renal angiography and selective clearance tests). Some investigators have, however, questioned the value of the renogram compared with that of excretion urography, for example (Dollery 1960, Poker et al 1960, Moses et al 1961). It is mainly the difficulty in interpreting the external renal curves in an objective and correct way that is responsible for the uncertainty and doubt regarding the value of the test. Evaluation of the curves by visual inspection alone or from different types of parameters calculated from the renogram have given widely divergent results.

¹³¹I labelled Hypaque dissolved in 1.5-4.7 ml sterile water (concentration 1.99-3.38 m_g/ml with a specific activity of 111-760 μCi/m_g). The purity of the radioactive preparation was checked by paper chromatography and was stated to be not less than 90 per cent (Abbott Laboratories claim).

In order to keep the amounts of radioactive substances used for renal scans and for intravenous infusions as uniform as possible the stock solutions were diluted with physiologic saline. A working solution (solution A) was obtained by adding 1.0 ml of 40 per cent non radioactive Hypaque (= 400 m_g) and adjusting the volume to 100 ml with sterile physiologic saline. The specific activity of Hypaque in this solution when used varied from 11.9 μCi/m_g to 3 μCi/m_g.

Hypaque solution B was prepared in the same way but with no addition of carrier. Specific activity of Hypaque in this solution when used varied from 25 μCi/m_g to 100 μCi/m_g.

The solutions were kept in glass bottles sealed with rubber membranes and stored up to 18 days in the dark at room temperature. Solutions not used within this time were discarded.

Purity and stability of ¹³¹I Hypaque solutions

All Hypaque solutions used by the author in clearance or renogram studies were routinely checked before and during use in the patients for any free

I by measurement of the 24 hour

thyroid uptake and by supplementary radiochemical analysis of randomly selected samples with high voltage paper electrophoresis.

On two occasions a significant 24 hour thyroid uptake was noted. On one of the occasions this prompted a more comprehensive analysis with high voltage paper electrophoresis in order to follow the liberation of iodide or other components during storage of this Hypaque solution.

Technique

The thyroid uptake was measured at 24 hours by the routine technique used in our isotope laboratory. This technique is essentially in accordance with that recommended by IAEA (1963). A solution of ¹³¹I Hypaque taken from the stock solution was diluted to a volume of 100 ml. This solution was used as a standard in the calculation of thyroid uptake.

High voltage paper electrophoresis was performed according to Wieland & Pfeleiderer (1955). Paper Whatman filter paper No. 1 MM. The buffer used was pyridine, glacial acetic acid, water (100:10:890) pH 6.0. Voltage about 1800 volts and potential gradient about 15 volt/cm. Temperature -8° to -10°C. Application 0.005-0.010 ml 8 cm from the cathode. Running time 60 minutes. The mobility of Hypaque was about 3 cm/1800 V/hour and that of iodide 18 cm/1800 V/hour. After separation the papers were dried at 40°C for 12 minutes. The iodine

RADIOCHEMICAL ANALYSIS OF ^{131}I -LABELLED HYPAQUE*General remarks*

Chemically sodium diatrizoate is 3,5-diacetamido 2,4,6 triiodo benzoic acid sodium salt ($\text{C}_{11}\text{H}_8\text{I}_3\text{N}_2\text{NaO}_4$) and its molecular weight is 635.9. Its structural formula is shown in Fig. 1. Hypaque is a white crystalline solid, which contains 59.87 per cent iodine. The iodine exists in a stable, organically bound state (Langecker et al. 1954, Knoefel & Huang 1956, McChesney & Hoppe 1957). The Hypaque salt is highly water soluble. Isolation and chemical analysis indicate that Hypaque is excreted unchanged in the urine (Langecker et al. 1954, McChesney & Hoppe 1957). Hypaque is synthesized and supplied by Winthrop Stearns Inc., New York, U.S.A., in solutions of varying concentration for use in diagnostic radiology, especially of the kidneys. The methylglucamine salt of diatrizoate (Renografin) has also been synthesized and is used as a contrast medium. A mixture of sodium and methylglucamine salt of diatrizoate in the proportion 10:66 is marketed as Urografin.

The non radioactive Hypaque (Hypaque 45 or 50 per cent) is a highly soluble white crystalline solid. Hypaque is relatively thermostable and tol-

erates autoclaving. Edathamercalium disodium 1:10,000 has been added as a stabilizer. The solution should be protected from strong light. Samples protected from light still comply with the control specification after six years' storage at room temperature. Check examination with paper chromatography and thin layer chromatography have not revealed any impurities (Winthrop Stearns Inc. pers. comm.).

 ^{131}I -labelled Hypaque

The radioactive Hypaque was supplied by Abbott Lab., Oak Ridge, Tenn., U.S.A. It was supplied as a sterile and pyrogen free solution in rubber stoppered glass vials. Each batch (stock solution) contained 2 mCi

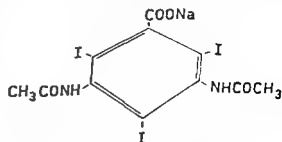


FIGURE 1 Sodium 3,5-diacetamido 2,4,6-triiodobenzoate

The chemical identity of the two components

Radioactive and non radioactive Hypaque were studied with high voltage paper electrophoresis to find out whether they behaved in the same way. The blackening of the autoradiograms in experiments using radioactive Hypaque coincided with the blue spots containing the non radioactive Hypaque.

Since radioactive Hypaque was prepared by an exchange reaction between non radioactive Hypaque and $\text{Na } ^{131}\text{I}$ it was thought that some radioactive iodide might persist in the radioactive Hypaque solution. When $\text{Na } ^{131}\text{I}$ was examined separately the radioactivity appeared as a single spot and it corresponded to the spot in the investigation of the Hypaque solution. Also the spot in autoradiograms of radioactive iodide corresponded to the spot in the investigation of the radioactive Hypaque solution. Thus the behavior of ^{131}I was similar to the corresponding component in the separation systems and autoradiograms of radioactive Hypaque solutions investigated.

Changes during storage of the solution

The changes in the composition of the preparation of radioactive Hypaque that had given a thyroid uptake of 7-8 per cent were followed by examining samples at different intervals. The results showed a further decrease of the Hypaque fraction (3-4 per cent) during 18 days storage and an almost corresponding simultaneous increase of the relative content of ^{131}I .

No new fractions were detected.

The reproducibility of the separation method was investigated in 6 estimations of the above mentioned solution. Mean value and standard deviation of the content of ^{131}I Hypaque in per cent of the total radioactivity 82.9 ± 0.5 .

Comments

The 24 hour thyroid uptake in clearance and renographic studies with different solutions showed that only on two occasions (all together three patients) did the thyroid uptake exceed 17 per cent indicating a higher degree of free iodide in the solution. No difference in uptake was noted between pure tracer solutions and solutions with addition of a small amount of non radioactive Hypaque. There was good agreement between the thyroid uptake values and the corresponding results obtained with high voltage electrophoresis. In the two preparations where the uptake was increased the occurrence of free iodide was verified.

The behaviour of ^{131}I was similar to that of the main impurity component found in the radioactive Hypaque solutions. It was thus very probable that this fraction was identical with ^{131}I . High voltage electrophoresis showed a decrease of the radioactive Hypaque by a further 3-4 per cent during 18 days storage.

The results obtained with various radioactive Hypaque solutions were in good agreement with the 24 hour thy-

containing compounds were developed according to Gmelin & Virtanen (1959). The localization of the radioactivity was determined by beta-ray scanning with an automatic windowless paper chromatogram scanner and by autoradiography with Guxaert Rapid X-ray films and an exposure time of 1–2 days.

Solutions

- (1) ^{131}I Hypaque solutions A and B (see page 17)
- (2) A water solution of carrier free ^{131}I with about the same radioactivity as solution B
- (3) non radioactive Hypaque (45 per cent Hypaque solution) was diluted with physiologic saline to a concentration of 0.450 mg/ml

Results

24 hour thyroid uptake

In the amounts of radioactive Hypaque (25–50 μCi) given in both the clearance and renographic studies the dose gave between 60 000 and 120 000 cpm when measured with the apparatus and technique used. The 24 hour thyroid uptake varied between 500–1000 cpm which corresponds to an uptake of about 0.4–1.7 per cent. The thyroid took up an equal amount of the two solutions (A and B) used. The thyroid uptake of two preparations exceeded the above limits (5 and 7–8 per cent respectively). These two preparations were therefore not used further. The high values found for free

iodide in these preparations were confirmed by high voltage paper electrophoresis.

High voltage paper electrophoresis

Fig. 2 shows the results of analysis with beta scanning of an electrophoretogram and an autoradiogram of a solution which had given a 24 hour uptake of 1.0 per cent in a patient. As can be seen, a good separation was obtained with this electrophoretic technique. The radioactive components are Hypaque (96.6 per cent), iodide (3.0 per cent) and unidentified impurity (0.4 per cent). Thus the two main components represented almost the entire activity, but some other irregular components sometimes occurred. They were, however, never found to exceed 1.0 per cent. The autoradiogram verified the existence of the two components in the electrophoretic paper studied. The solutions which had given 24 hour thyroid uptakes of 5 and 7–8 per cent in patients, contained 10 and 16–17 per cent free radioactive iodide. The corresponding values of radioactive Hypaque were 90 and 83–84 per cent in analysis with high voltage paper electrophoresis.

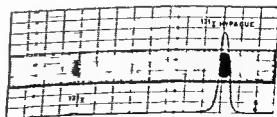


FIGURE 2 High voltage paper electrophoresis of fresh stock solution of radioactive Hypaque. Autoradiogram and scan curve of the paper strip. S, starting point.

CHAPTER V

METHODS

Determination of radioactive amounts given

The following procedure was used for estimating the radioactivity of the amount given. Different amounts of radioactive Hypaque solution (0.2—0.8 ml) were drawn up in 20 tuberculin syringes. The syringe was placed and counted in the symmetry line of a scintillation detector with a collimator diameter of 63 mm. The distance between the crystal and the aperture of the collimator was 110 mm and that between the aperture and the syringe 140 mm. The content was then ejected into a volumetric flask. The volume was made up to 1000 ml with water and 20 ml was taken for counting in a well type scintillation detector. A good linear relation was found between the two counting series and a factor for converting count rate of the syringe to the count rate of the well type detector could be calculated.

Standards

0.10 ml of the stock solution of ^{131}I labelled Hypaque was removed with a syringe and its radioactivity measured

under the scintillation detector. The content was ejected into a glass flask containing 1000 ml of water. 20 ml of this solution was counted daily in the well scintillation counter. This solution was used as a standard in the examination of blood, urine and bile samples.

Measurement of activity in blood, urine and bile samples

The samples were counted in a well scintillation detector with a $2'' \times 1\frac{7}{8}''$ thallium activated iodide crystal and a $1\frac{1}{2}'' \times \frac{3}{8}''$ central well capable of receiving a tube containing a 5 ml sample. The detector was mounted in a lead shield and connected to a scaler. The capacity of the recording system of the scaler was tested by measuring radioactivities of varying strength. A linear relationship was found between amount of radioactivity and the pulse rate up to 100,000 cpm. The background was always measured before and after the series of sample were studied. The blood and bile samples were counted for 30 minutes and the urine samples for 10 minutes or as the time for a predictor

roid uptake found in patients in a previous study (Denneberg et al 1961a), where the uptake was less than 10 per cent, and in animal studies using autoradiography (Denneberg et al 1960).

Blaufox et al (1963d) found 5 per cent free iodide and about 5 per cent unidentified impurities in commercially available radioactive Hypaque. They thought these impurities to be at least partly responsible for the dif-

ference found between Hypaque and creatinine clearance in dogs. In animal experiments Kimbel & Borner (1955) found no significant radioactivity over the thyroid gland of the rat 24 hours after injection of ^{131}I Urografin, while zum Winkel & DeMura (1961) reported an uptake of 0.41–0.47 per cent by the thyroid gland after 200 minutes. The importance of checking the purity of the radioactive solution is discussed in Chapter VII F.

METHODS

Determination of radioactive amounts given

The following procedure was used for estimating the radioactivity of the amount given. Different amounts of radioactive Hypique solution (0.2–0.8 ml) were drawn up in 20 tuberculin syringes. The syringe was placed and counted in the symmetry line of a scintillation detector with a collimator diameter of 63 mm. The distance between the crystal and the aperture of the collimator was 110 mm and that between the aperture and the syringe 140 mm. The content was then ejected into a volumetric flask. The volume was made up to 1000 ml with water and 20 ml was taken for counting in a well type scintillation detector. A good linear relation was found between the two counting series and a factor for converting count rate of the syringe to the count rate of the well type detector could be calculated.

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The samples were counted in a well scintillation detector with a $2'' \times 1\frac{7}{8}''$ thallium activated iodide crystal and a $1\frac{1}{2}'' \times \frac{5}{8}''$ central well capable of receiving a tube containing a 5 ml sample. The detector was mounted in a lead shield and connected to a scaler. The capacity of the recording system of the scaler was tested by measuring radioactivities of varying strength. A linear relationship was found between amount of radioactivity and the pulse rate up to 100,000 cpm. The background was always measured before and after the series of sample were studied. The blood and bile samples were counted for 30 minutes and the urine samples for 10 minutes or as the time for a predictor

mined number of counts (100,000). The standard deviation of the blood, bile and urine samples counts was always less than 1.5 per cent. The stability of the apparatus was checked daily by measuring the activity of a ^{22}Na standard.

Chemical methods

Para aminohippuric acid (PAH) was determined largely by the method of Brun (1951). 1 g of p dimethylamino benzaldehyde, 50 ml of acetic acid, and 50 ml of physiologic saline were mixed. 5 ml of this reagent was added to 0.100 ml of native serum. Suitable dilutions of the urine were prepared

before analysis. A 24 hour urine sample was diluted 1:50—1:200 and 0.100 ml of the diluted urine was taken. The reaction was read either immediately or several hours later at a wave length of 465 nm in a Zeiss PM Q II spectrophotometer against blanks taken before infusion of PAH. Standard solutions of PAH of 4 mg/100 ml were run in parallel. The reaction was carried out at room temperature. The error of a single determination (36 calculated from 36 double determinations) was 1.06 per cent.

Inulin was determined by the method of Heyrowsky (1956) with slight modifications (Dennberg et al 1961). The error of a single determination (36 double determinations) was 1.57 per cent.

DISTRIBUTION AND ELIMINATION OF ^{131}I LABELLED HYPAQUE

Introduction

The animal experiments showed a rapid elimination of Hypaque or Urografin from the blood by the kidneys and no substantial uptake in other organs and no signs of any significant extrarenal excretion.

Knowledge of the kinetics of Hypaque is necessary for evaluating the suitability of the substance for clearance tests with the single injection technique. This chapter is concerned with the blood concentration curves, the excretion of the test substance in the urine and bile as well as the distribution volume of ^{131}I Hypaque in a clinical series. The formulae given in the appendix for three different types of clearance tests with the single injection technique have been applied in the clinical series and comparisons have been made between the results obtained by the single injection and intravenous infusion technique.

I Material

Clearance studies were performed on 31 hospital patients including 15 with and 17 without diseases related to the kidneys. These patients included 21 in whom clearance was also studied by

the intravenous infusion technique and the isotope renogram (Chapters VII and VIII). The initials, sex, age, body surface, haematocrit, plasma volume and the clinical diagnosis are given in Table I. The purpose of the investigation was not to assess the normal limits of the Hypaque clearance with a single injection of Hypaque but to compare the values obtained by different types of clearance tests in patients with and without impaired renal function. In two patients who had recently been subjected to cholecystolithotomy biliary excretion was studied in bile collected from indwelling T-tubes. In addition in 17 patients with and 15 without renal disease the excretion of the test substance in the urine was determined and separate analyses were made of venous blood. These patients are also included in the material examined with the isotope renograms (Chapter VIII).

II Technique

The patients were instructed to drink as much as they could before the test. They were examined in the semirecumbent position.

Table 1 Clinical material (31 cases) used in study of clearance of Hypaque with single injection technique

Case	Sex	Age (years)	Height cm	Weight kg	BSA m ²	Her %	PV ml	Diagnosis
IN	M	57	174	87	2.0	37	3760	Cephalalgia
IH	M	49	172	55	1.7	39	3040	Lumbar disc degeneration
SP	M	41	172	74	1.9	10	3430	Rheum arthritis
KA	M	32	183	70	1.9	47	3610	I pilepsy
DA	M	60	171	56	2.0	11	3790	Humero-scapular periarthritis
WJ	M	53	174	83	2.0	14	3220	Spondylosis deformans
LL	F	50	159	11	1.1	37	2450	Cholelithiasis
HB	F	33	155	48	1.4	46	2350	I pilepsy
AJ	M	35	170	71	1.8	15	3040	Neurasthenia
LA	M	22	181	72	1.9	39	3470	Rheum arthritis
SN	M	61	170	76	1.9	46	2960	Rheum arthritis
SA	M	42	167	65	1.7	41	2690	Neurasthenia
HN	F	54	161	61	1.7	37	2340	Cephalalgia
ML	M	22	185	80	2.1	44	3490	Nephropathy (Haematuria)
AO	M	53	174	73	1.9	36	3670	Proteinuria + Haematuria
RA	M	59	185	78	2.0	37	2600	Polycystic kidneys + Nephrolithiasis
BL	F	34	164	55	1.6	35	2670	Ess hypertension
BA	M	52	173	75	1.9	46	3120	Nephropathy (Proteinuria)
GA	M	49	170	65	1.8	39	3170	Prostatic hypertrophy
HJ	M	20	190	100	2.3	40	4220	Nephropathy (Chronic glomerulonephritis)
HG	F	55	160	61	1.6	30	2470	Nephropathy (Proteinuria)
YS	M	49	174	73	1.9	47	3040	Ess hypertension
WA	F	61	161	48	1.5	37	2480	After left-sided nephrectomy + Diabetes mellitus
IB	F	34	157	76	1.5	40	2510	Nephropathy
VA	M	60	183	65	1.9	34	3690	Horseshoe kidneys
KG	F	30	162	66	1.7	38	2590	Ess hypertension
KA	F	29	163	54	1.6	42	2400	Bilateral malformation
AO	M	53	180	66	1.8	26	1050	Chronic pyelonephritis Nephrolithiasis
LV	M	34	168	65	1.7	27	1720	Nephropathy (Abuse of phenacetin)
TB	M	65	173	56	1.7	23	3780	Chronic pyelonephritis
BF	M	37	164	62	1.7	23	3560	Chronic glomerulonephritis

Injection of ¹²⁵I-Hypaque 0.2–1.0 mCi (0.4 μ Ci/kg body weight) of solution A was given *in vivo*

Sampling and methods

Blood. With a catheter inserted in the left brachial artery samples were

collected in 9 heparinized tubes (5 ml) in the course of 3–120 minutes. After litemolysis 2 ml was taken for counting. All measurements of the whole blood were corrected with 2 per cent for uptake of radioactive Hypaque by the red blood cells (Danchette *et al.* 1961) which means that the activity

measured was calculated as the plasma concentration of ^{131}I Hypaque.

In the series of 28 other patients where the venous blood was analysed these samples were collected 60 and 90 minutes after the injection.

Urine The patient voided urine before and 120 minutes after the injection. As a rule the ur. ex. voided during the rest of the day (24 hours) was collected (Table X). The 2 hour urine sample was diluted 1:10 and 2 ml was used for counting. 24 hour samples were used undiluted.

Bile was collected from the 1 tube during the 0-10, 30-60 and 60 minute 24 hour periods after the injection. Undiluted samples of 2 ml each were used for counting.

Haematocrit In the analysis in 80 mm long tube was centrifuged for 15 minutes (3000 rpm) in a centrifuge of type MSE haematocrit.

Plasma volume The plasma volume was determined from sex, height and weight with the aid of Gibson's nomogram (Gibson et al 1937). In a few cases it was determined directly with Evans blue (Ford 1951).

The technique used for the studies of clearance with intravenous infusion technique is described in Chapter VII.

Calculations

The appendix (page 97) summarizes the kinetic problems associated with the single injection technique as well as the analysis of the clearance of substances eliminated entirely or partly by the kidneys. The general formulae are also given for the calculation of

total plasma and renal clearance (with and without correction for renal dead space delay) and of the distribution volume.

The initial blood activity (cpm/ml) was calculated from the amount of radioactivity injected (cpm) and total blood volume. All the subsequent values found were expressed as a fraction of this and shows the proportion of the amount of radioactivity still in the circulating blood.

Slope analysis of the curve on semi-logarithmic paper was started from the end of the curve according to the standard procedure (Miles 1963, page 146). As a rule 3 or 4 exponential functions of time were obtained. The intercepts and slopes (calculated according to the formula $b = \ln 2/T_{1/2}$ where $b = \text{slope}$ and $T_{1/2} = \text{half time}$) were read and the total area was calculated according to the principles described in the appendix.

For the 2 hour renal clearance the correction for the renal dead space delay was calculated from the urine flow after which the corresponding area under the plasma curve was determined by numerical or graphical integration (see appendix). The urine values (cpm/ml) from 2 and 24 hour tests respectively are expressed as a fraction of the amount of radioactivity injected. The total clearance was calculated according to formula No. 1, the 24 hour renal clearance according to No. 2, and the 2 hour renal clearance according to No. 3. The distribution volume was calculated according to formula No. 4 with the aid of the intercepts and the slopes.

Calculation of the clearance by the constant intravenous infusion technique is given in Chapter VII

The clearance values used in this chapter were not corrected to hold for 1.73 m² body surface

Retention per cent The retention of ¹³¹I-Hypaque in the blood was calculated from both the arterial and the venous curves. This parameter consists of the quotient between the 90 minute value and the 60 minute value $\times 100$. The retention per cent is compared with other renal function data for ¹³¹I Hypaque (clearance respectively urinary recovery) in this chapter (see below) and in Chapter VIII it is given in relation to parameters calculated from the external chest and renal curves

C Results

Blood curve Fig. 3 a gives a scatter diagram of the blood values in the 13 controls with the mean concentration curve (broken). The curve falls first steeply and then gently. After 5 minutes the curve has fallen to 35 per cent, after 10 minutes, to 25 per cent, after 1 hour to 11 per cent and after 2 hours to 7 per cent. The individual curves are still slightly concave in the semilogarithmic scale. Table IX shows the calculated intercepts and exponential constants for the 13 controls.

Fig. 3 b shows the blood curves for the 18 patients with renal disease. For natural reasons the individual curves differed in shape. The series also in-

cludes normal curves. This is because the series consisted of patients with signs of renal disease but without manifest impairment of renal function. At 5 minutes the values lay between 25 and 52 per cent, and at 10 minutes, between 20 and 42 per cent. The slope successively became more gentle in patients with impaired renal function. One hour after the injection the values were between 9 and 26 per cent, and after 2 hours between 5 and 22 per cent. Neither in this group were the individual curves linear at 120 minutes. Table IX gives the intercepts and exponential constants for these 18 cases.

Excretion in urine The excretion values noted at 2 hours in the 13 controls were 51, 54 and 62 per cent (mean value 56 per cent). The summe value was obtained for 8 patients with a clearance of more than 100 ml/min in the infusion clearance test. At 24 hours 95 per cent (observed range 86—101 per cent) had been excreted by 23 controls. This figure may be somewhat too low owing to incomplete collection of voided urine. One control excreted 2 per cent and another 3 per cent during the second day. In the patients with renal diseases the values were lower although in some of them the urinary recovery was high. The results of urinary recovery (2 and 24 hours) are given in graphical and tabular form (Figs. 6 and 7, Table X).

Excretion in the bile In the two controls where the excretion of the bile was followed after a single injection of ¹³¹I Hypaque the 30 minute, 60 minute and 24 hour values noted

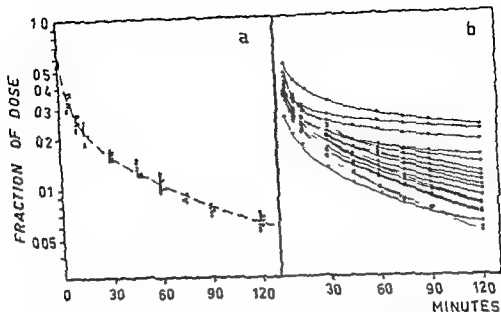


FIGURE 3a Blood concentration of Ilypaque in 13 controls expressed as fraction of dose given and mean concentration curve (broken) 1 Curves for blood concentration of Ilypaque in 18 cases with renal disease

were 0.01, 0.03, 0.13 per cent in one and 0.03, 0.04 and 0.22 per cent in the other.

Clearance correlations

Table 3 gives the clearance with the intravenous infusion technique and the total 2- and 24-hour renal clearances and in Figures 4a, b and c these three clearances are plotted against the clearance with infusion technique. The figures show a good correlation between clearance with the infusion technique and those with single injection technique ($r = 0.93$ and 0.93 respectively, 0.97). The total clearance, however, differs markedly from the other two in that the majority of the values are above the 45° line. The cal-

culated regression line confirms that the values for the total clearance are systematically higher and above the 45° line $y = 0.97x + 21.3$ (where y = total clearance and x = clearance with the infusion technique). Also the 24-hour renal clearance showed a similar picture with most of the cases above the 45° line but this tendency was much less marked than that noted for the total clearance. The regression line was $y = 1.009x + 3.6$ where y = 24-hour renal clearance. The smallest deviation was shown by the 2-hour renal clearance; the values were crowded around the 45° line and the regression line ($y = 0.972x + 0.7$ where y = 2-hour renal clearance) deviated only slightly from the identity line.

Calculation of the clearance by the constant intravenous infusion technique is given in Chapter VII

The clearance values used in this chapter were not corrected to hold for 1.73 m^2 body surface

Retention per cent The retention of ^{131}I Hypaque in the blood was calculated from both the arterial and the venous curves. This parameter consists of the quotient between the 90 minute value and the 60 minute value $\times 100$. The retention per cent is compared with other renal function data for ^{131}I Hypaque (clearance respectively urinary recovery) in this chapter (see below) and in Chapter VIII it is given in relation to parameters calculated from the external chest and renal curves

C Results

Blood curve Fig. 3 a gives a scatter diagram of the blood values in the 13 controls with the mean concentration curve (broken). The curve falls first steeply and then gently. After 5 minutes the curve has fallen to 35 per cent, after 10 minutes to 25 per cent, after 1 hour to 11 per cent, and after 2 hours to 7 per cent. The individual curves are still slightly concave in the semilogarithmic scale. Table IX shows the calculated intercepts and exponential constants for the 13 controls.

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Excretion in the bile In the two controls where the excretion of the bile was followed after a single injection of ^{131}I Hypaque the 30 minute, 60 minute and 24 hour values noted

the blood within 2 hours. The initial segment falls rapidly also in cases with renal insufficiency but the later decline of the curve is much slower than in the controls. This is apparent on visual examination as well as on comparison between the exponential constants of the curves.

The rapid fall in the blood concentration was in good accord with that found by Schlunghauß & Bilton (1956b) with ^{131}I labelled Urografin and by Brunch (1961), Dencker et al (1961a) and Stokes & Ter Poorten (1964) with ^{131}I labelled Hypaque. The rapid elimination of the substance from the blood corresponds to the rapid excretion in the urine with more

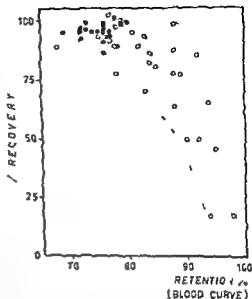


FIGURE 6 Relationship between 24 hour urinary excretion and retention of Hypaque calculated from blood curve (33 cases). Demarcation lines of the field are based on observed values. Solid circles Controls. Open circles Cases with renal disease.

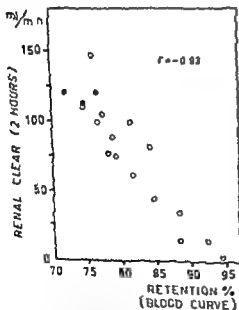


FIGURE 7 Relationship between 2 hour renal clearance and retention of Hypaque calculated from blood curve. 33 cases. Solid circles Controls. Open circles Cases with renal disease.

than 50 per cent in 2 hours, 90 per cent in 24 hours and 97–98 per cent in 48 hours. These values agree with those found by Schlunghauß & Bilton (1956b) who reported 60 per cent in 1 hour and 90 per cent in 24 hours. Similar results have been published by Kumbel & Horner (1955) and Langer et al (1956) who found 24–34 per cent in 1 hour and 27–44 per cent in 2 hours while their 24 hour values were much lower (14–63 per cent). No explanation was however offered for the low level of these values. It is clear from our results that practically all ^{131}I Hypaque is eliminated by the kidneys in other words

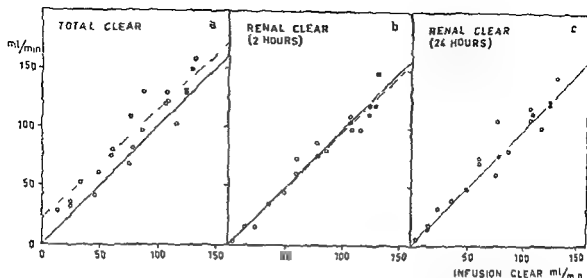


FIGURE 4 a Total clearance plotted against clearance of Hypaque with infusion technique (21 cases) b 2 hour renal clearance plotted against clearance of Hypaque with infusion technique (18 cases) c 24 hour clearance plotted against clearance of Hypaque with infusion technique (20 cases) Uninterrupted line 45° line Broken line Calculated regression line Solid circles Cases without renal disease Open circles Cases with renal disease The clearance values are not corrected to hold for 1.73 m² body surface

Comparison between retention per cent and 2-hour renal clearance and urinary recovery

Fig 5 gives the 2 hour renal clearance plotted against retention per cent in 18 cases. The figure shows a negative correlation between the values ($r = -0.93$) i.e. the greater the retention the lower the clearance.

The 24 hour urinary recovery is plotted against retention in 53 cases in Fig 6 which shows a non linear relation between these variables. A similar relation was found between the urinary recovery (2 respectively 24 hours) and the 2 hour renal clearance in 25 and 18 cases, respectively (Fig 7). The figure shows that the 2 hour recovery per cent varies more closely with clearance than the 24 hour recovery, the latter recovery often being high in cases with impaired renal function.

The limits given in Figs 6 and 7 are based on observed and not on calculated values.

Distribution volumes

Table X gives the distribution volume for 31 cases. The mean for the 13 controls was 13.4 litres with an observed range of 8.6 to 18.3 litres which corresponds to 20 respectively 13–28 per cent of bodyweight. The 18 patients with renal disease had a mean value of 13.6 litres with an observed range of 10.6 to 18.6 litres and 20 and 13–28 per cent respectively of bodyweight.

D Discussion

The results show that the normal plasma curve for ¹³¹I Hypaque rapidly falls after a single injection and more than 90 per cent of the activity leaves

the blood within 2 hours. The initial segment falls rapidly also in cases with renal insufficiency but the later decline of the curve is much slower than in the controls. This is apparent on visual examination as well as on comparison between the exponential constants of the curves.

The rapid fall in the blood concentration was in good accord with that found by Schlunbaum & Billion (1956b) with ^{131}I labelled Urografin and by Bianchi (1961), Denneberg et al (1961a) and Stokes & Ter Pogossian (1964) with ^{131}I labelled Hypaque. The rapid elimination of the substance from the blood corresponds to the rapid excretion in the urine with more

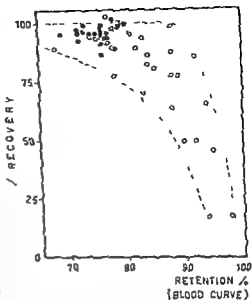


FIGURE 6 Relationship between 24 hour urinary recovery and retention of Hypaque calculated from blood curve (23 cases). Demarcation lines of the field are based on observed values. Solid circles: Controls. Open circles: Cases with renal disease.

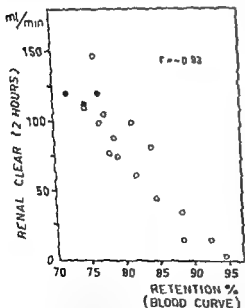


FIGURE 7 Relationship between 2 hour renal clearance and retention of Hypaque calculated from blood curve (18 cases). Solid circles: Controls. Open circles: Cases with renal disease.

than 50 per cent in 2 hours, 93 per cent in 24 hours and 97–98 per cent in 48 hours. These values agree with those found by Schlunbaum & Billion (1956b) who reported 60 per cent in 3 hours and 90 per cent in 24 hours. Similar results have been published by Kimbel & Börner (1953) and Langacker et al (1954) who found 24–54 per cent in 1 hour and 27–44 per cent in 2 hours, while their 24 hour values were much lower (54–65 per cent). No explanation was however offered for the low level of these values. It is clear from our results that practically all ^{131}I Hypaque is eliminated by the kidneys; in other words,

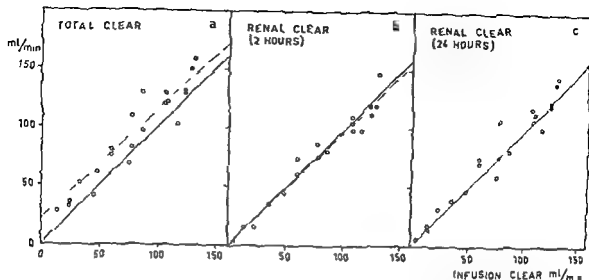


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D Discussion

The results show that the normal plasma curve for ^{131}I Hypaque rapidly falls after a single injection and more than 90 per cent of the activity leaves

and 23.0 per cent for both sexes together. Brinchi (1961) reported somewhat higher values for the distribution volume of ^{131}I Hypaque (25—30 per cent) but he gave no details about the method used. It is possible that he used the formula intercept/final slope which according to Riggs (1963) gives artificially high values.

The retention per cent was calculated mainly as a simple measure of retention of Hypaque in the blood. Since the parameter is calculated as a quotient of the values obtained from two separate blood analyses it avoids the otherwise necessary repeated sampling. The good relation with renal clearance, urinary recovery and retention showed that retention may be taken as a measure of the total renal function and consequently supplement other renal function data. In Chapter VIII retention calculated from the blood curve is compared with other parameters calculated from external chest and renal curves and thereby provides a possibility of studying the relation between external and internal isotope measurements.

On comparison between the clearance with the intravenous infusion technique and total respectively 2 and 24 hour renal clearances the 2 hour renal clearance was found to be the best of the three clearances with the single injection technique. The total clearance systematically showed artificially high values. This may be explained by the fact that the plasma curve had not reached its final slope because Hypaque had not yet attained its final distribution. This means that

the area obtained was too small and the total clearance according to formula 1 was too high. To use the total clearance of Hypaque the final slope should therefore be calculated from a curve covering a longer period e.g. 5 hours in analogy with the studies of Hypaque distribution volume. On comparison between the 2 and 5 hour total clearance values in the above mentioned five cases the 5 hour values were 7—11 ml/min lower than those noted at 2 hours.

The calculation of the 2 hour renal clearance according to the formula II did not give too small an area under the curve because an observed limited part of the total area under the plasma curve was used. The factors which must be considered are the radioactivity in the renal dead space at the end of the period of urine collection and the magnitude of the urine flow during the clearance period. These factors and the corrections for transport and mixing delay respectively are discussed in the appendix (page 97). It is clear from Table X that in most of the patients urine flow was acceptable only in 3 of the 18 was it below 1.0 ml/min. In 2 of these cases the correction for total delay was considerable (10 minutes) while the remaining had between 4.3—6.9 minutes.

The results show that the 2 hour renal clearance after a single injection can be used instead of the clearance with the infusion technique of ^{131}I Hypaque. It also appears that renal 24 hour clearance can be used while the total clearance based on the values

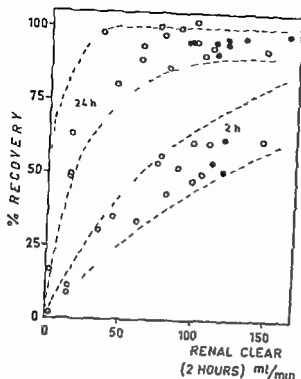


FIGURE 7 Relationship between 2 and 24 hour urinary recovery and 2 hour renal clearance of Hypaque (18 and 20 cases). Demarcation lines of the two fields are based on observed values. Solid circles Controls. Open circles Cases with renal disease.

extrarenal excretion is insignificant in normals.

The low values found for excretion of ^{131}I -Hypaque in the bile are in agreement with results obtained by Kimbel & Borner (1955), who found a small amount of radioactivity in the duodenal juice 40 minutes after the injection. They also demonstrated a slight activity in the gastric contents, but none in the saliva. As to the faecal excretion, Schlunbaum & Billon (1956b) found 1–2 per cent radioactive Urografin 48 hours after the injection. It should also be mentioned that the uptake by the thyroid (see Chapter IV) was low, usually below 1 per cent (0.4–1.7 per cent). These

data show that ^{131}I Hypaque (or ^{131}I Urografin) is normally excreted almost quantitatively by the kidneys.

In the present investigation, partly for practical clinical reasons, the plasma curve was not followed for more than 120 minutes, which appeared to be sufficient. Earlier preliminary clearance studies have given good results within such an interval (Danneberg et al. 1961a). Many substances require a much longer time for the plasma curve to reach its true final slope. If sampling is stopped before the curve has reached its final slope and if the slope of the last segment obtained is extrapolated to infinity, the total distribution volume calculated from formula 6 will be artificially small (see appendix). The shorter the period that the plasma curve is followed the larger the error. Examination of controls with Hypaque for 5 hours showed that the values for distribution volume calculated from this long curve were larger than the corresponding 2 hour values. Thus the value found for five normals was on the average 2.4 litres or 3.4 per cent higher for 5 hours than for 2 hours. The latter values are minimum values for the Hypaque distribution volume. The values obtained from the 5 hour curve imply that the distribution volume should be increased from 20 to 23.4 per cent of bodyweight which value is exactly the same as the bromide space. Moore et al. (1963) found the bromide space (after correction for uptake of bromide by red blood cells) to be 23.9 per cent of the bodyweight for males, 22.0 per cent for females.

THE RENAL EXCRETION MECHANISM OF ¹³¹I-LABELLED HYPAQUE

Introduction

The investigations on animals and on humans have given divergent results regarding the renal excretion mechanism of Hypaque. The possibility of a partial tubular secretion and/or reabsorption could not be excluded. Since no experiments had been performed with inhibitors of tubular reabsorption, the question was not definitely cleared up. To study the mechanism of renal excretion of Hypaque and especially the tubular secretion clearance studies were performed with the infusion technique and simultaneous administration of inulin, PAH and radioactive Hypaque with and without blocking of the tubuli with Probenecid.

A. Material

The material which consisted of 60 patients was divided into three series (A, B, C). The age distribution of the series are given in Table II.

Series A consisted of 29 patients with various diseases not related to the kidneys. Laboratory studies revealed no signs of renal disease in these patients. They were normotensive and afebrile. The initials, sex, age, height, weight, body surface area and diagnoses are given in Table III.

Series B consisted of 22 patients who

had been admitted to the medical department on one or more occasions because of diseases affecting the kidneys or for investigation of suspected renal disease. Initials, sex, age, height, weight, body surface area and diagnoses are given in Table IV.

Series C consisted of 9 female patients including one with proteinuria (chronic glomerulonephritis?), 2 with essential hypertension and 6 with diseases unrelated to the kidney. The initials, sex, age, height, weight, body surface area and diagnoses are given in Table V. The influence of Probenecid was tested in this series.

Series A served as a control series in comparative clearance studies with various substances. Series B represented a group of patients with renal disease with varying degree of renal insufficiency. Series A and B were studied under identical conditions while series C was examined under varying conditions after injection of Probenecid.

Series A was divided into four subgroups according to the composition of the infusion solution: A 1 the first 18 patients in Table II, A 2 the following three patients (G, J, R, C, M, N), A 3 four patients (U, S, V, H, A, D, I, S), A 4 four patients (M, P, A, P, G, S, C, J).

from a 120 minute curve did not give correct clearance. On the other hand the total clearance calculated from a plasma curve followed for a long time seems to provide a possible method but from a clinical point of view such a long examination period is disadvantageous. The advantage of total clearance is that the determination is based entirely on the plasma values. No collection of urine is necessary and all risks of errors due to incomplete collection are thereby avoided. Single injection clearance has several advantages over clearance with the intravenous infusion technique: it requires no infusion aggregate, it requires no equilibration period and enables simultaneous evaluation of the total renal function and renography.

The greatest disadvantage from a practical clinical point of view is however, the necessity of arterial sampling. This has been considered necessary because of the arteriovenous difference in the concentration of the substance with falling plasma values after a single injection (Brun et al 1949b, Smith 1951). As is apparent

from the appendix (page 97), it is probable that the formulae given (1 and 3) can be used for venous blood if the blood curves are followed for a long time, e.g., one day. An alternative to arterial sampling would be the use of capillary samples, but then the amount of ^{131}I labelled Hypaque injected would have to be increased.

Finally, it should be emphasized that the single injection clearance can only be used for substances whose clearance rate is independent of the plasma concentration. Thus when Diodrast is used, the proportion taken up by the red blood cells and that bound by protein result in markedly falling clearance values at low plasma concentration (Hilden 1946, Hogeman 1948, Newman et al 1949, Block & Burrows 1960). As far as Hypaque is concerned the uptake by the red blood cells is small (Denneberg et al 1961a) the protein binding is insignificant (Lisser et al 1962) and the results of clearance with the intravenous infusion technique suggest no variation of clearance with the plasma level (Chapter VII).

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Introduction

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Table II Survey of material used in investigation of renal excretion mechanism of Hypaque

Series	No of cases			Age (years)	
	Total	Female	Male	Range	Mean
A	29	11	18	21—69	44.7
B	22	7	15	22—63	48.0
C	9	9		21—64	46.1
Total	60	27	33	21—69	46.0

Table III Controls used in study of clearance of Hypaque with intravenous infusion technique

Series	Case	Sex	Age (years)	Height cm	Weight kg	BSA m ²	Diagnosis
A 1	MA	F	41	157	93	1.9	Psychoneurosis
	UA	F	44	158	60	1.6	Neurasthenia
	LO	F	49	148	53	1.5	Rectal cancer
	DS	F	51	175	114	2.3	Gastric ulcer
	BJ	M	24	173	59	1.7	Rheum arthritis
	JL	M	24	178	79	2.0	Cephalgia
	AG	M	25	170	58	1.7	Tonsillitis
	SH	M	26	181	70	1.9	Angiolipoma of skin
	AB	M	41	179	75	1.9	Cephalalgia
	SP	M	41	172	74	1.9	Rheum arthritis
	IH	M	49	172	58	1.7	Lumbar disc degen
	SL	M	50	171	77	1.9	Rheum arthritis
	NS	M	51	179	87	2.1	Cerebral a—v aneurysm
	GZ	M	54	178	99	2.2	Epilepsy
	LN	M	57	174	87	2.0	Cephalalgia
A 2	BK	M	61	169	70	1.8	Glaucoma
	NK	M	64	171	85	2.0	Neurasthenia
	JJ	M	65	173	62	1.7	Gastric ulcer
	GJ	F	28	168	61	1.7	Psychoneurosis
	RE	F	33	165	44	1.5	Gastritis
A 3	YN	F	38	165	66	1.7	Rheum arthritis
	US	F	25	155	40	1.3	Depression
	VH	F	43	166	56	1.6	Cephalalgia
	AD	F	64	170	81	1.9	Angina pectoris
	IS	M	69	167	66	1.8	Pulm emphysema
A 4	MP	F	35	167	54	1.6	Psychoneurosis
	AP	M	43	168	53	1.6	Psychoneurosis
	GS	M	49	170	70	1.8	Psychoneurosis
	CJ	M	52	184	67	1.9	Cephalalgia

Table IV Some data about cases of renal disease used in study of clearance of Hypaque with intra venous infusion technique

Series	Case	Sex	Age (years)	Height cm	Weight kg	BSA m ²	Diagnosis
II	OD	M	44	170	74	1.9	Myelopathy + Monia vesic. urin
	BL	F	34	164	55	1.6	Ess hypertension
	IL	M	53	185	62	1.8	Rheum heart disease + Proteinuria
	HL	M	22	185	80	2.1	Nephropathy (Haematuria)
	AO	M	53	174	76	1.9	Proteinuria + Haematuria
	BA	M	52	173	78	1.9	Nephropathy (Proteinuria)
	JA	M	49	172	58	1.7	Prostatic hypertrophy
	HA	M	59	185	78	2.0	Polycthemia vera + Nephrolithiasis
	FA	M	61	175	87	2.0	Prostatic hypertrophy
	WA	F	64	161	48	1.5	After left sided nephrectomy + Diab mell
	GA	M	49	170	65	1.8	Prostatic hypertrophy
	HG	F	55	160	61	1.6	Nephropathy (Proteinuria)
	AS	M	49	174	73	1.9	Ess hypertension
	HH	F	56	161	60	1.6	Scleroderma
	HJ	M	29	190	100	2.3	Nephropathy (Chronic glom nephritis?)
	FD	F	54	157	76	1.8	Nephropathy
	AS	M	60	183	65	1.9	Horseshoe Kidneys
	IC	F	30	162	66	1.7	Ess hypertension
	HA	F	29	168	54	1.6	Bilat renal malformation
	AO	M	55	180	86	1.8	Chronic pyelonephritis + Nephrolithiasis
	TD	M	65	173	56	1.7	Chronic pyelonephritis
	IA	M	34	168	65	1.7	Nephropathy (Abuse of phenacetin)

Table V Some data about cases used in study of effect of Probenecid

Series	Case	Sex	Age (years)	Height cm	Weight kg	BSA m ²	Diagnosis
I	BI	F	21	156	60	1.6	Epilepsy
	BN	F	30	161	66	1.7	Proteinuria
	AI	F	31	168	73	1.8	Cephalalgia
	UN	F	40	170	68	1.8	Otitis externa
	IC	F	45	159	48	1.5	Rheum arthrit.
	AW	F	56	158	49	1.5	Rheum arthrit
	RI	F	63	161	72	1.8	Arthrosis deformans
	RII	F	64	160	71	1.8	Ess hypertension
	AI	F	56	164	82	1.9	Ess hypertension

B Technique

Substances

Pyrogen free inulin in sterile 50 ml ampoules containing a 10 per cent solution (Laevosan Gesellschaft, Germany) was used. Para aminohippuric acid was supplied in sterile 50 ml ampoules containing a 20 per cent solution of the sodium salt (Sharp and Dohme U.S.A.). Non radioactive Hypaque in a 15 per cent solution in sterile 20 ml ampoules was used (Winthrop, England). The radioactive solution used is described in Chapter IV.

Composition of the infusion solution

The infusion solution contained about 0.75 per cent inulin, 0.60 per cent para aminohippuric acid and 0.079 per cent non radioactive Hypaque in 600 ml saline.

A varying amount of radioactive Hypaque (0.20–1.80 ml) from solution A (see Chapter IV) was added to the infusion solution. This amount was adjusted to the weight of the subjects (0.6–1.2 $\mu\text{Ci/kg}$ bodyweight which corresponded to 30–80 μCi). Of the radioactive dose one fifth was given in the priming dose and four fifths in a drip solution. This produced a plasma concentration of 1–4 mg PAH, 10–20 mg inulin and 1–2 mg Hypaque per 100 ml plasma.

This infusion solution was given to all the patients but with the following exceptions and modifications.

Series A 2 Three cases in this series were given radioactive Hypaque from

the stock solution (see Chapter IV) without any addition of non radioactive Hypaque. The concentration of non radioactive Hypaque in the infusion solution was about 0.0002 per cent and the calculated plasma concentration of the substance was extremely low (0.002–0.003 mg/100 ml plasma).

Series 1 3 To the four cases in this series radioactive Hypaque was given from the stock solution (see Chapter IV). In addition non radioactive Hypaque was added in such quantities as to obtain a final concentration of 0.040 per cent non radioactive Hypaque in the infusion solution which produced a plasma concentration of 0.6–0.8 mg/100 ml.

Series A 4 In the four cases in this series a method was used which was identical with conventional clearance tests. The labelled material was added to sufficient 15 per cent stable Hypaque to maintain a constant blood level at, or just below, 6 mg/100 ml plasma during the clearance determination. The concentration of non radioactive Hypaque in the infusion solution was 0.3 per cent.

Series B In this series of patients with renal diseases the concentration of PAH, inulin and non radioactive Hypaque was varied according to the impairment of renal function in those with renal insufficiency. In 8 of 20 patients in this series no PAH was given in the infusion solution.

The total amount of radioactive Hypaque given during the test (= radioactivity of Hypaque in the priming dose + radioactivity from the amount

included in the continuous infusion) varied between 30 and 50 μCi . The plasma concentration of PAH, inulin and non radioactive Ilypaque in the pathological series (series B) was of the same order and within the same limits as in the control series (series A 1).

Examination procedure

The patients were in the fasting state but had been instructed to drink as much fluid as possible before the test to obtain a good urine flow. The test was performed in the morning and took 2–2 1/2 hours. During the infusion tests the patient was recumbent.

Apparatus and performance of infusion

The substances were administered in a priming dose followed by intravenous infusion at a constant rate. An infusion pump was used which permitted the administration of the solution at 4 ml/min in 10 minutes after which the infusion was given by means of the drip method by which the rate was standardized (3–4 ml/min). The first clearance period was started about 40 minutes after the beginning of the drip infusion. Three 20–30 minute clearance periods were used (1, 2, 3).

In the experiments with Probenecid in series C the examination period was somewhat longer than in series A because of supplementary clearance periods (2 1/2–3 hours). On the other

hand the individual clearance periods were somewhat shorter (15–20 minutes). After 2 initial clearance periods Probenecid solution was given intravenously for 10–15 minutes. The solution was in sterile 100 ml ampoules of 1 per cent (Probenecid sodium solution)¹. The doses given varied between 500 and 2200 mg. After the end of the Probenecid injection a new clearance period was started and 3 further periods were studied.

Sampling

Blood Samples of blood (8–10 ml per sample) were drawn from an antecubital vein 5 respectively 15 minutes after the beginning of each clearance period.

Urine The urine samples were collected with an indwelling catheter. During urine collection periods the catheter was allowed to drain into a glass. About two minutes before the end of each period the bladder was emptied as completely as possible by suprapubic pressure. Residual urine was removed by instillation of 50 ml sterile saline. Sometimes after the urine had been expelled 50 ml of air was introduced into the bladder and expelled in order to remove the last washout fluid. The total volume of the fluid (= urine plus washout fluid) was measured.

Comments on clearance experiments

In one of the cases (B 5) the patient reported nausea and diffuse abdominal pain and cold sweat during admini-

¹ Probenecid solution was generously supplied by AB Astra.

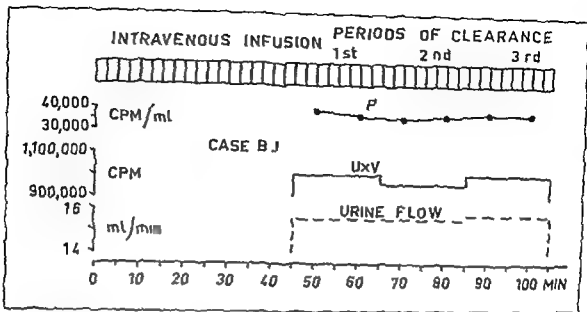


FIGURE 8 Clearance of Hypaque with intravenous infusion technique used and calculated clearance values in B J (control) P Plasma sample (cpm/ml) U Urine sample (cpm/ml) V Urine volume (ml)

¹³¹ I Hypaque clearance (ml/min)	135	131	135
Inulin clearance (ml/min)	116	126	117
PAH clearance (ml/min)	669	763	672
Quotient Hypaque/inulin clearance	1.16	1.06	1.15
Quotient Hypaque/PAH clearance	0.20	0.18	0.20
Filtration fraction (Inulin/PAH clearance $\times 100$)	17	17	17

stration of Probenecid, and a moderate transient decrease in the blood pressure was noted. But no severe signs of shock were registered. In the remaining patients Probenecid produced no side effects.

C Treatment of data

Clearance calculations

For each period 20 ml undiluted plasma, 20 ml undiluted urine (from the urine plus washout fluid) and 20 ml diluted standard (from the infusion solution) were counted (see Chapter V). The standard used was diluted 1:10. The plasma concentration of

Hypaque was calculated by the formula $\frac{\text{serum sample cpm}}{\text{standard cpm}} \times \text{concentration of Hypaque (mg/ml) in infusion solution}$. ¹³¹I Hypaque clearance (C_{Hyp}) was calculated by inserting cpm/ml urine and plasma directly into clearance formula $C_{Hyp} = \frac{U \times V}{P}$, where U = cpm/ml of urine, V = ml of urine per min and P = cpm/ml plasma. The plasma level was calculated as the arithmetic mean of the two blood samples collected at the beginning and the end of each period. The clearance of paraaminohypuric acid (C_{PAH}) and inulin (C_{in}) were calculated by the method

$$\frac{\text{urine volume (ml)} \times \text{urine ext} \times \text{dilution}}{\text{serum extinction}}$$

The clearance values were then corrected to hold for 1.73 m² body surface area.

In the above mentioned clearance experiments the standard performance according to Smith (1951) with a priming dose with increased concentration of PAH was not used. The main purpose of the examination was to study the excretion mechanism of Hypaque and to avoid the risk of any simultaneous competition effect between Hypaque and PAH the latter substance was given in such quantities that the plasma level was relatively low.

After an equilibration period of 45—50 minutes the plasma level of the radioactive Hypaque in half of the cases was stable during the entire test period while in the other half (especially in cases with impaired renal function) the plasma level increased slowly by 10—12 per cent during the entire experimental period. This implies an increase of about 3—4 per cent per period. This increase was accompanied by a moderate increase in the secretion of urine. The results were not corrected for increase in blood concentration or increase of the urine output. Correction would be justified because rising plasma levels give increasing clearance (Brun et al. 1949 b) and changes in delay time (Bojesen 1951 a). The reasons why no correction was made in the present investigation were: 1) in the appraisal of a quotient between the clearances of the two substances which were determin-

ed simultaneously, the effect of the above changes in the blood concentration were insignificant. 2) In these clearance experiments urine flow was good and correction for changes in delay time was therefore of less importance.

D Results

¹³¹I Hypaque clearance

Tables VI, VII and VIII give the initials of the subjects in series A and B, the haematocrit number and duration of clearance period, urine flow, clearance of Hypaque, inulin, PAH, quotient Hypaque/inulin clearance, filtration fraction $\times 100$ and the mean of the 3 clearance periods and of the quotients as well as the means after correction to hold for 1.73 m² body surface area.

The mean value for Hypaque clearance in the entire control series (A) was 115 ml/min with an observed range of 80—153 ml/min. The mean for the females was 116 ml/min and for the males 114 ml/min. In the 3 series (1, 2, 3, 4) where different doses of non radioactive Hypaque were added to the radioactive solution the values were of the same order as in the larger series (A, 1).

In the series with renal diseases (B) the clearance was 15—117 ml/min. That clearance values fell within the normal range in this pathological series may be explained by the fact that it included cases with clinical signs of renal disease but without

manifest impairment of renal function

Inulin and PAH clearance

In the control series (A 1) the mean inulin clearance was 102 ml/min with an observed range of 71–130 ml/min and that of the PAH clearance 498 ml/min with a range of 316–708 ml/min (in 3 cases the inulin clearances were not calculated for technical reasons and in 2 cases the PAH clearance was not studied)

In series B the clearance of inulin and PAH in cases with renal disease sometimes fell within the same range as in the controls. The inulin values ranged from 13 to 113, and those for PAH from 32 to 620 ml/min (in 8 of the cases PAH clearance was not studied)

Age distribution

To study the effect of age on renal function the material (series A) was divided into 3 groups: 20–39, 40–49, 50–69 years. The mean values found for ^{131}I Hypaque clearance were 127 ml/min (9 patients) in the 1st age group, 110 ml/min (9 patients) in the 2nd group and 108 ml/min (11 patients) in the 3rd group. The corresponding values for inulin were 116 ml/min (3 patients), 105 ml/min (5 patients) and 93 ml/min (7 patients) and for PAH 610 ml/min (4 patients), 491 ml/min (6 patients) and 432 ml/min (6 patients). The material is distributed according to age in Fig. 9. It is clear from the diagram that the clearance

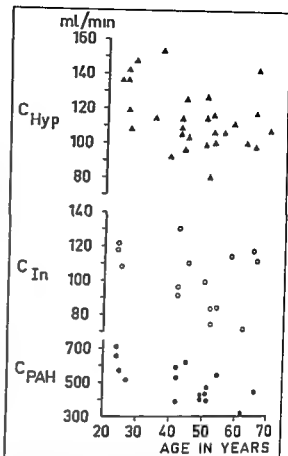


FIGURE 9 Mean clearance of Hypaque, inulin and PAH in relation to age in controls (Series A 1). C_{Hyp} Hypaque clearance, C_{In} inulin clearance, C_{PAH} PAH clearance. The clearance values are corrected to hold for 1.73 m² body surface.

of all 3 substances varied with the patients' ages.

Quotient Hypaque/inulin clearance

The quotient Hypaque/inulin clearance was on the average 1.17 (45 periods) in the control series (A 1) with an observed range of 0.63–1.86. The corresponding mean quotient in the material for the patients studied with Probenecid (18 periods before Probenecid injection) was 1.19 with an

observed range of 0.89—1.40. The mean in series B was 1.20 (65 periods) with an observed range of 0.64—2.26 and for the total material a mean value of 1.21 (128 periods). In the statistical test (Student's *t* test) the Hypaque/inulin quotient differed significantly from 1.0 ($P < 0.05$) in all three series.

Filtration fraction (Inulin/PAH clearance $\times 100$)

The mean filtration fraction was 20 (39 periods) in the control series (A 1) and 23 (41 periods) in series B with observed ranges of 14—28 and 10—30 respectively.

Quotient Hypaque/PAH clearance

The quotient Hypaque/PAH clearance in the control series (A 1) was 0.24 (48 periods) with an observed range of 0.13—0.47 and in series B it was 0.31 (41 periods) with an observed range of 0.13—0.81.

Comparison between ^{131}I Hypaque and inulin clearance

In Fig. 10 a the clearance of Hypaque in series A 1 is plotted against that of inulin. It is clear that the majority of values were above the identity line except for the periods with high inulin clearance when the values were

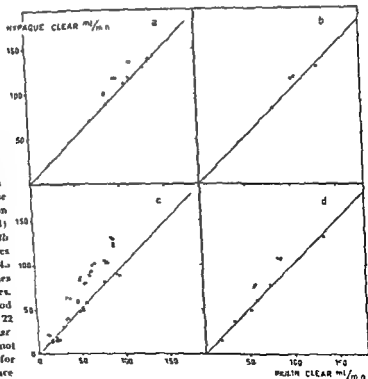


FIGURE 10 Relation ship between Hypaque and inulin clearance in 1) controls (Series A 1) and 2) cases with renal disease (Series B). a) Series A 1 40 period values. b) Series A 1 10 mean values. c) Series B 60 period values. d) Series B 22 mean values. The clearance values are not corrected to hold for 1.73 m² body surface.

below the 45° line. The scatter diagram thus illustrates the above mentioned finding that the mean quotient Hypaque/inulin clearance exceeded 1.0 in the series. In the diagram 35 of 45 values are on or above the 45° line.

Fig. 10 c shows the same tendency in series II with 49 of 65 values on or above the 45° line.

Figs. 10 b and d give the mean values (of three periods) for the two series. These scatter diagrams show

the same tendency as those for the individual periods, but with much narrower scatter of the values.

Comparison between quotient Hypaque/inulin clearance and inulin clearance

Figs. 11 a—d show the quotients and mean quotients Hypaque/inulin clearance plotted against inulin clearance.

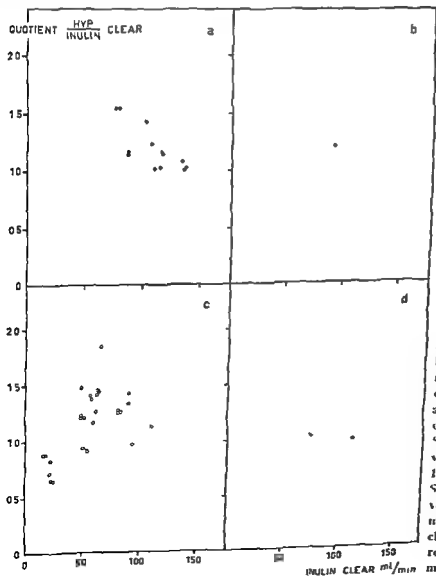


FIGURE 11 Relation ship between quotient Hypaque clearance and inulin clearance in controls (Series A 1) and cases with renal disease (Series B) a Series A 14 period values b Series A 14 mean values c Series B 22 period values d Series B 22 mean values Inulin clearance is not corrected to hold for 1.73

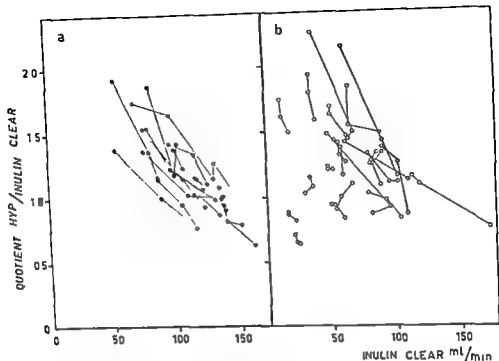


FIGURE 12 Relationship between quotient Hypaque/inulin clearance and inulin clearance in 15 controls (Series A) and 22 cases with renal disease (Series B). Period values in individual cases joined by lines. a Series A b Series B

(period values and mean values for three periods) in series A and B.

In Fig. 11a the negative correlation between the Hypaque/inulin clearance quotient and the inulin clearance suggests that the larger the inulin clearance the smaller the quotient. The quotient decreases to 1.0 and some times even to a lower value when the inulin values are high. In series B this relationship is not so distinct because of the wider scatter of the values. This scatter in the pathological series may be due to the quotient values being artificially large at low clearance and therefore less suitable for analysis of this type.

Fig. 11b shows the same linear relationship for the mean values as for the individual periods while Fig. 11d like Fig. 11c shows a less distinct tendency of the mean values in the pathological series.

Figs. 12a and b give the quotient Hypaque/inulin clearance plotted against the inulin clearance where the period values for the respective patients are connected by lines to enable evaluation of the individual values in every case. The relationship found for the entire series holds also for the individual cases. The increase in the inulin clearance usually causes a decrease in the quotient.

below the 45° line. The scatter diagram thus illustrates the above mentioned finding that the mean quotient Hypaque/inulin clearance exceeded 1.0 in the series. In the diagram 35 of 45 values are on or above the 45° -line.

Fig. 10 c shows the same tendency in series B with 49 of 65 values on or above the 45° -line.

Figs. 10 b and d give the mean values (of three periods) for the two series. These scatter diagrams show

the same tendency as those for the individual periods, but with much narrower scatter of the values.

Comparison between quotient Hypaque/inulin clearance and inulin clearance

Figs. 11 a—d show the quotients and mean quotients Hypaque/inulin clearance plotted against inulin clearance.

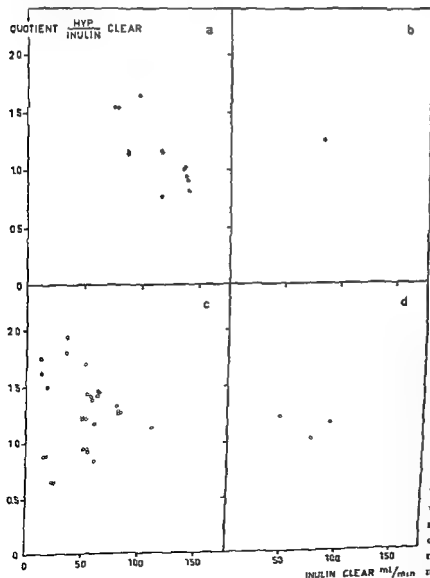


FIGURE 11 Relation ship between quotient Hypaque clearance and inulin clearance in controls (Series A 1) and cases with renal disease (Series B). a Series A 1 45 period values b Series A 1 15 mean values c Series B 65 period values d Series B 22 mean values. Inulin clearance is not corrected to hold for 1.73

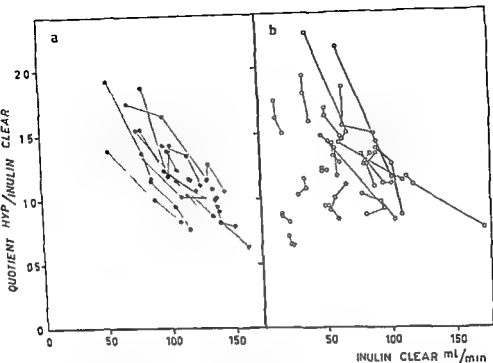


FIGURE 12 Relationship between quotient Hypaque inulin clearance and inulin clearance in 10 controls (Series A 1) and 22 cases with renal disease (Series B). Period values in individual cases joined by lines. a Series A 1 b Series B

(period values and mean values for three periods) in series A 1 and B.

In Fig. 11 a the negative correlation between the Hypaque/inulin clearance quotient and the inulin clearance suggests that the larger the inulin clearance the smaller the quotient. The quotient decreases to 10 and some times even to a lower value when the inulin values are high. In series B this relationship is not so distinct because of the wider scatter of the values. This scatter in the pathological series may be due to the quotient values being artificially large at low clearance and therefore less suitable for analysis of this type.

Fig. 11 b shows the same linear relationship for the mean values as for the individual periods, while Fig. 11 d like Fig. 11 c shows a less distinct tendency of the mean values in the pathological series.

Figs. 12 a and b give the quotient Hypaque/inulin clearance plotted against the inulin clearance where the period values for the respective patients are connected by lines to enable evaluation of the individual values in every case. The relationship found for the entire series holds also for the individual cases. The increase in the inulin clearance usually causes a decrease in the quotient.

Studies with Probenecid (Series C)

Table XIV gives the initials, mg Probenecid given, number and duration of clearance periods, urine flow, Hypaque and inulin clearances and quotients *Hypaque/inulin clearance*

In this series interest was focused mainly on the changes in the quotient *Hypaque/inulin clearance* following the administration of Probenecid. As is apparent from the table, cases B N V W and E C differed from the remainder in that they showed no depression of the quotient *Hypaque/inulin clearance* after administration of Probenecid. As described on page 37, B N was the only patient who had discomfort during the examination. The other two cases had rheumatoid arthritis, which had been

treated with silicic drugs. These three cases were not included in Figs 13 and 14.

The remaining 6 cases showed different degrees of depression of the quotient *Hypaque/inulin* after administration of Probenecid. It should be observed that in R P and U N the concentration of inulin was not quite steady during the first two periods, so that the quotient *Hypaque/inulin clearance* was not quite constant. In the remaining cases the concentration of both inulin and Hypaque were satisfactory during the examination.

During the first two periods (before the injection of Probenecid) the Hypaque clearance was higher than the inulin clearance with the exception of the 2nd period in cases R P and U N. During the last 3 periods (after injection of Probenecid) all inulin values were higher than the corresponding Hypaque values with the exception of case A N where the inulin values in the 3rd and 5th period were lower than the Hypaque values. The time of the maximum depression effect on the quotient *Hypaque/inulin* varied somewhat, but in most cases it fell in the 4th period.

In view of the relation between the quotient *Hypaque/inulin clearance* and inulin clearance changes in filtration (inulin clearance) during the experiment must be taken into account in the investigation of the effect of Probenecid.

In Fig. 13 the Hypaque clearance in the 6 patients is plotted against inulin clearance. Their period clearance values are connected by lines.

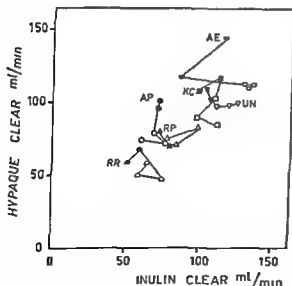


FIGURE 13 Hypaque clearance plotted against inulin clearance in study of Probenecid in 6 cases (Series C). Period values in individual cases joined by lines. Solid symbols indicate clearance values before and open symbols after injection of Probenecid.

which enable individual evaluation of the clearance series

Fig 14 gives the quotient Hypaque/inulin clearance plotted against the inulin clearance and here too the period clearance values are connected by lines. It is clear from the two figures and Table XIV that in some of the cases the inulin clearance increased after the injection of Probenecid which *per se* can result in a lowering of the Hypaque/inulin clearance quotient. In two of the cases (A E and U N) the decrease of the quotient was not larger than what might be explained by this increase in inulin clearance. In the other 4 cases where the inulin clearance was largely unchanged the quotient decreased even to values below 1.0. Case II N was difficult to judge because of the side effects of the drug.

In patients V W and L C who received salicylic acid the inulin clearance was possibly decreased after the injection of Probenecid. The quotient was not significantly changed in these tests but was if anything somewhat increased in the 4th period while the others showed maximal depression.

E Discussion

The amount of radioactive Hypaque in clearance tests was calculated per kg bodyweight (0.6–1.2 $\mu\text{Ci/kg}$). The total radioactivity during the test varied between 30–50 μCi . These doses give sufficiently high counting rates to ensure accuracy in the determination of the radioactivity. The

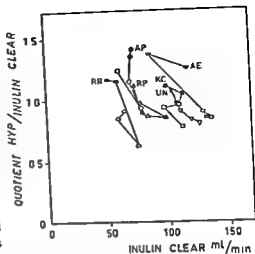


FIGURE 14 Quotient Hypaque/inulin clearance plotted against inulin clearance in study of Probenecid in 6 cases (Series C). Period values in individual cases joined by lines. Solid symbols indicate clearance values before and open symbols after injection of Probenecid.

radioactive dose delivered to the gonads was so small as to imply no risk to the patients according to experimental experience and data obtained with ^{131}I labelled Urografin reported by Feine & Bruer (1961). A dose of 0.2 $\mu\text{Ci/kg}$ bodyweight for 24 hours implied a whole body dose of 18 mrad which they considered had been reduced to one third by rapid elimination of the substance during the first few hours. 14 μCi for 24 hours implied a gonadal dose of 9–16 mrad in experimental phantom experiments and Feine & Bruer (1961) concluded that these doses found were much lower than those in excretion urography for example.

The clearance of radioactive Hypaque at various plasma levels was of

the same order, but the series were too small to allow any statistical analysis. It was therefore not possible to draw any definite conclusions regarding differences between the series. Other investigators who used tracer doses with extremely low serum levels of Hypaque, also reported clearance values of the same order. Branchi & Zampiere (1961) gave 0.6–0.9 $\mu\text{Ci/kg}$ body weight to 13 normal subjects with the intravenous infusion technique and obtained values of 86–188 ml/min with a mean value of 116 ml/min. Denneberg et al. (1961), who gave radioactive Hypaque with a small addition of non radioactive Hypaque, found that in contrast to radioactive Diodrast, the clearance of Hypaque did not change at the serum levels used. Morris et al. (1965) found no difference in clearance between tracer doses of ^{131}I Renografin alone and combined with 60 ml of 60 per cent non radioactive Renografin.

In clearance studies with ^{131}I labelled contrast media the purity of the radioactive solution must be considered. The occurrence of free radioactive iodide with its clearance of about 31 ml/min might be expected to give lower clearance values as shown by Burbank et al. (1961) in a comparison between ^{131}I labelled Hippuran and PAH. No comparisons have been made between the clearance of radioactive and non radioactive Hypaque. In the present investigation the radioactive preparations used contained more than 95 per cent radioactive Hypaque. These should give somewhat lower clearance values than non radioactive

Hypaque. For clinical use contamination of this order can be ignored as long as the percentage of free radioactive iodide in the preparation is known.

The mean mulin clearance in the control series was lower than in that described by Goldring et al. (1940). This was probably because of the considerable overrepresentation of elderly patients in the present series (50 per cent above 50 years). Davies & Sholk (1950) reported a decrease in the clearance rate of mulin and Diodrast clearance with age. The mulin clearance found in the present series fell within the observed and statistical limits ($\bar{M} + 2\text{SD}$) found in their material. The results obtained with ^{131}I Hypaque and PAH also showed a similar decrease of renal function with age as judged from the mean values in the three age groups.

According to Lasser et al. (1962) the protein binding of Hypaque in man is negligible. This means that most of the Hypaque can be regarded as freely filtrable in the glomerulus. The question whether Hypaque is eliminated not only by filtration but also by tubular secretion was elucidated by comparison between Hypaque and mulin clearance. This comparison showed that although the values crowded around the 45° line and were closely related the majority of the Hypaque values were above the identity line in the scatter diagram. This means that the mean quotient Hypaque/mulin clearance in the material is a whole was larger than 1.0.

According to Smith (1951) it may

be accepted that the substance studied is excreted by the tubules in addition to being filtered through the glomeruli if the clearance of any substance studied which is not synthesized by the kidneys, is greater than the simultaneous inulin clearance in man i.e. when the substance studied/inulin clearance is greater than 1.0

Studies with the tubular blocking substance Probenecid showed that in those cases where the inulin clearance was largely unchanged a clear decrease occurred in the quotient. This must be explained as a direct effect of Probenecid on the secretion of Hypaque.

Why then does the quotient Hypaque/inulin vary inversely with the glomerular filtration rate (inulin clearance)? Assuming that Hypaque is freely filtrable and tubular secretion is fairly constant at different filtration rates the quotient will be higher at low filtration while a higher filtration will give a lower quotient but still above 1.0. If this quotient falls below 1.0 filtration alone plus secretion cannot explain this relationship but reabsorption must be assumed. Such a quotient below 1.0 was found at high inulin rates (see Fig. 11) and after blocking with Probenecid which not only lowered the quotient to 1.0 but also below this value.

What is the interaction between these 3 mechanisms and what is the reabsorption at different filtration rates? It is possible that at low filtration reabsorption is also low while at high filtration reabsorption is high which would thus mean that reabsorption is constantly proportional

to the filtration. Such a mechanism would be in accord with Bojesen's (1954b) theory of the salt and water reabsorption in the tubules of the mammalian kidney. Bojesen (1954b) showed that as far as the excretion of water is concerned it is possible that tubular reabsorption capacity determines the order of the filtration and that there is a proportional relation between the filtration and the reabsorption. This explains why the quotient Hypaque/inulin at low filtration exceeds 1.0 (when tubular secretion is greater than reabsorption) and at high glomerular filtration rate below 1.0 (when tubular secretion is less than reabsorption).

These observations thus suggest tubular reabsorption of Hypaque. This seems to explain the delay of the initial excretion of Hypaque compared with inulin which has been reported in studies using the single injection technique (Denneberg et al. 1961a).

It should however, be pointed out that this possible reabsorption could not be determined quantitatively from available data. It is reasonable to assume that estimations based on the depression of the Hypaque/inulin quotient below 1.0 in the Probenecid studies must lead to corresponding low values. There are two reasons for this: it was not possible to use maximum doses of Probenecid because of side effects and it is not certain that Probenecid can block tubular secretion completely.

The renal excretion mechanism of Hypaque (Ioprosin or Renoprosin) has been studied in animal experi-

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These observations thus suggest tubular reabsorption of Hypaque. This seems to explain the delay of the initial excretion of Hypaque compared with inulin which has been reported in studies using the single injection technique (Dennberg et al 1961a).

It should however be pointed out that this possible reabsorption could not be determined quantitatively from available data. It is reasonable to assume that estimations based on the depression of the Hypaque/inulin quotient below 1.0 in the Probenecid studies must lead to corresponding low values. There are two reasons for this: it was not possible to use maximum doses of Probenecid because of side effects and it is not certain that Probenecid can block tubular secretion completely.

The renal excretion mechanism of Hypaque (Urografin or Reno-grafin) has been studied in animal experi-

ments with different methods (Chapter II) These investigations have given divergent results It was found that glomerular filtration dominated, while the question of possible tubular secretion and also the possibility of re-absorption was left unanswered In 13 normal subjects Bianchi & Zampieri (1961) found that the quotient between radioactive Hypaque and thiosulphate clearance was 0.91 ± 0.15 with an observed range of 0.74—1.21 Bianchi & Toni (1963) reported a comparison between radioactive Hypaque and inulin in subjects with and without renal diseases They found a good correlation between the clearance of the two substances, but they give no details In their scatter diagram 16 of the 35 observed values were above the identity line (i.e. quotient Hypaque/inulin was above 1.0) 4 along the line and 15 below the line

Turesson et al (1964) reported similar results with inulin and radioactive Hypaque clearance, with only a narrow scatter around the 45° line They examined 47 patients with suspected renovascular hypertension and found a mean difference of 2.2 ml/min per 1.73 m^2 between these substances with a correlation coefficient of 0.99 and a mean Hypaque/inulin quotient of 0.96

Recently Morris et al (1965) compared ^{125}I labelled Renografin and inulin on 13 subjects with and without renal disease They found a mean Renografin/inulin quotient of 1.04 and an observed range of 0.76—1.86 (69 clearance periods)

No explanation can be offered for the difference between the results in

the abovementioned investigations and the present study Only at high glomerular filtration rate was agreement found with the previous investigations Of the factors responsible for too low a Hypaque/inulin clearance quotient in the investigations referred to above, it is possible that one was a higher degree of impurity of the radioactive preparation and the other a competition effect of some other substance (e.g. PAH) in the infusion solution Only Morris et al (1965) have given data on the purity of the preparation (2 per cent free radioactive iodide in the solution), while Turesson et al (1964) reported that despite various techniques, their results were inconclusive when labelled diatrizoate was assayed for purity This might indicate that they used a preparation with a high percentage of free iodide and other impurities In a previous investigation on dogs they had found 5 per cent iodide plus 5 per cent non identified impurities and they reported this as a cause of the difference found between Hypaque and creatinine clearance (Blaufox et al 1963 d)

It should be pointed out that the comparison between Hypaque and inulin clearance shows the net result of all processes involved in clearance mechanism and not the individual processes acting in different directions (tubular secretion and re-absorption) The fact that a substance has a clearance identical or nearly identical with that of inulin is no proof that it is excreted by glomerular filtration only This has been shown by Lindahl & Josephson (1945) with the substance

sulpha methyl thiodiazole. The essential thing is not whether the quotient Hypaque/inulin clearance is 1.0 but whether it is depressed and falls after administration of a tubular blocking substance. When it does it is a sign of tubular secretion of the substance.

Radioactive Hypaque and Renografin have been tried experimentally as a suitable substance for the single injection clearance (Bianchi 1961; Dennberg et al 1961a; Blaufox et al 1963d; Meschan et al 1963a) and as a substitute for inulin in the measurement of glomerular filtration by the intravenous infusion technique (Traux et al 1964; Morris et al 1965). The results obtained in the present inves-

tigation show that Hypaque is eliminated mainly by glomerular filtration while the tubular secretion and reabsorption more or less compensate one another. This means that even if Hypaque does not give a true measure of glomerular filtration it should be clinically useful as a substitute for inulin until more suitable substances have become available. Hypaque is also a suitable substance for the single injection clearance because the clearance appears to be independent of the plasma level. This indicates that the Hypaque clearance by the single injection technique is possible despite simultaneous tubular secretion and reabsorption.

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THE RADIOISOTOPE HYPAQUE RENOGAM QUANTITATIVE EVALUATION AND RESULTS IN RENAL DISORDERS

Introduction

As pointed out in Chapter II E, the choice of the most suitable ^{131}I -labelled substance for renography offered considerable problems. The advantages and disadvantages of the various substances made themselves felt in both experimental and clinical comparisons. Promising clinical results with the three radioactive diatrizoate products (Hypaque, Renografin, Urografin), were reported by Denneberg & Heden skog (1959 a), Winter et al (1959), Denneberg (1959/60), Bauer & Feine (1960), Abt & Balkus (1961), Grisser & Hawliczek (1962), Montandon et al (1962), Grisser et al (1963), Koecke & Bauer (1963), Staehler et al (1963) and others.

A considerable problem common to methods using ^{131}I labelled substances for renography is the difficulty in interpreting and obtaining quantitative measures of renal function from the curves.

The purpose of the investigation described in this chapter was to elucidate the clinical application of the renogram in a series of patients with

and without renal disease. On the basis of a theoretical analysis of the complex external renal curves, simple parameters were selected to allow quantitative evaluation of the renogram and definition of criteria of normal and abnormal curves.

A Material

The material is summarized in Table VI with respect to disease, number of patients, sex, age (range and means) and number of renograms and roentgenographic examinations (see also Table XX).

Series 1 Controls

This series consisted of ten healthy students and 21 hospital patients with diseases unrelated to the kidney and cardiovascular system and being treated for at the medical and surgical departments Malmö general hospital. None of the patients in this series had clinical or laboratory evidence of renal disease. They were normotensive and afebrile.

Table VI Survey of material used in renogram studies

Series	No of cases			Age (years)		No of	
	Total	Female	Male	Range	Mean	steno grams	roentg exam
A Control	31	3	28	17-57	33.2	41	
B Chronic glom. nephritis	8	5	3	30-49	39.1	8	2
C Chronic pyelonephritis	18	15	3	19-65	48.2	18	13
D Polycystic disease	4	1	3	21-44	35.0	4	4
E Other parenchymal diseases	18	6	12	19-60	41.1	18	14
F Hypertension	10	6	4	30-67	47.1	10	10
G Nephrectomy or aplasia	6	4	2	17-64	47.5	11	2
H Acute ureteral obstruction	33	13	20	14-72	42.0	33	32
I Hydronephrosis	13	5	8	33-69	52.0	31	13
Total	141	58	83	14-72	41.9	151	90

Series B Chronic glomerulonephritis

The eight patients in this series had been admitted on several occasions to the medical department for investigation and treatment of chronic glomerulonephritis which in six of the eight cases started after angina tonsillaris with acute glomerulonephritis. In two of the cases a transient nephrotic syndrome had preceded the chronic stage. Post mortem examination was performed in seven of these eight cases and in all of them the clinical diagnosis was confirmed.

Series C Chronic pyelonephritis

The 18 patients in this series had a history of several attacks of acute pyelonephritis and later chronic pyelonephritis. In five cases (V L & T & I & O & W W) there was a history of roentgenographically verified urinary obstruction and renal stone, while the others had no history of renal stone. Some cases also had roentgenographic signs of chronic pyelo-

nephritis satisfying the criteria given by Dejdar (1959). The majority of cases had impairment of renal function and clinical signs of chronic pyelonephritis at the time of renography. In nine of the 18 cases the clinical diagnosis was verified at autopsy.

Series D Polycystic renal disease

Three of the patients in the series had polycystic renal disease in their family history and the clinical diagnosis was verified at autopsy. In the fourth case (K P) there was no family history but the diagnosis was verified at surgical exploration.

Series E Other parenchymal diseases

This series of 18 patients was more heterogeneous in respect to the causes of the diseases than the preceding series. The patients had been admitted to the medical department for investigation of parenchymal renal disease with varying pathological findings (proteinuria, haematuria, bacteriuria,

impairment of renal function of obscure origin) and were cured for under the diagnosis of chronic nephritis or nephropathy of obscure origin. In several of the cases the diagnosis was suspect quiescent pyelonephritis, but since these cases did not fill the criteria of pyelonephritis they were assigned to this series. In three of the cases the patients had coexisting diabetes mellitus. Autopsy or laparotomy was performed in three of the 18 cases.

Series F Hypertension

This series consisted of ten patients with manifest hypertension, without previously known renal parenchymal disease. All the patients had been admitted on at least one occasion for extensive investigation and care but no endocrine disease or coarctation of the aorta could be demonstrated. A diagnosis of hypertension required demonstration of a diastolic blood pressure above 95 mm Hg and a systolic blood pressure above 150 mm Hg at rest (the values are based on the diurnal blood pressure recordings during spells in hospital) and ocular fundi of hypertensive type of at least grade II according to Keith, Wagener & Barker (1939). In two of the cases (Ö N and J G) the clinical diagnosis was verified at autopsy where bilateral nephrosclerotic changes were found. J G and Å N were the only patients who had a malignant hypertension with a short history and rapid progression, while the remaining eight patients showed the clinical picture of benign hypertension.

Series G Aplasia or post nephrectomy state

Two of the six patients in this series had at previous examination been found to have only one kidney, while the remaining four had undergone nephrectomy for various indications (hydronephrosis, unilateral silent kidney, pyelonephritis). In one of the cases with renal aplasia the clinical diagnosis was verified post mortem.

Series H Acute ureteric obstruction (suspect or certain)

The series comprised 33 patients referred to the surgical department because of suspect acute renal obstruction. After clinical examination they were referred for renography, which was done immediately, and then for excretion urography. Nine patients had been given spasmolytics before or on admission to the surgical department because of severe acute pain, and 19 had pain at the time of the examination (renography or urography).

Series I Hydronephrosis

This series consisted of 13 patients with hydronephrosis of varying origin. The series was much more heterogeneous in respect of origin than that of acute ureteric obstruction. Some of the patients were examined because of suspect renal stone with previous characteristic pain in their histories; some had previously known renal stone and had become worse and were to be examined and if considered necessary offered operation; some had a silent kidney and prostatic cancer.

and some had gynaecological cancer which was operated upon and treated with radiation. Common to the entire series was the fact that all of them had unilateral or bilateral roentgenological changes suggestive of hydronephrosis.

Of the cases with renal disease 86 were cared for at the medical department, 41 at the surgical department (including 33 ambulatory), two at the department of gynaecology, two at the department for infectious diseases (see appendix).

Some of the cases accounted for here have been presented in previous publications (Denneberg & Hedenskog 1959 b, Denneberg 1959/60), namely L A Ö P V P H H and the cases in the series of acute ureteric obstruction except G B T A and G R.

B Technique

Test substance

^{131}I labelled Hypaque was used in the roentgram studies. The composition and dilution of the radioactive solution (solution A) used in all cases except those in series II which were examined with solution B are given in Chapter IV.

The radioactive dose injected was 0.4 $\mu\text{Ci/kg}$ bodyweight. Preparation of standards and handling of the stock solutions is described in Chapter V.

Equipment for external measurement of radioactivity

Two scintillation detectors with a $1''$ by $1''$ thallium activated sodium iodide

crystal were used for measurements over the kidneys. The scintillation crystals were placed in a cylindric lead shield with a conic collimator with an inner diameter of 50 mm and an outer diameter of 65 mm. The distance between the crystal and the opening of the collimator was 35 mm and the angle of the collimator insert was 110° (Fig. 16). The two detectors were mounted on a stand which allowed adjustment in the horizontal and the vertical planes. The arrangement is illustrated in Fig. 15.

A third scintillation detector in a cylindric lead collimator with a channel length of 110 mm and collimator opening 60 mm in diameter was used for external measurements over the heart. The detectors were connected to ratemeters which were coupled to a 6 point recorder with fixed paper speed of 75 cm per hour. The 6 point recorder had a speed of 5 dots/minute and curve (chest curve or one of the two renal curves). The time constants could be set at 0.5, 2.5, 10 and 40 seconds.

Preparation of the patients and roentgenographic localisation of the kidneys

The patient was examined at the roentgen diagnostic department in the morning on the examination day for marking of the site of the kidney. The patient was instructed to drink before the examination and the only exceptions to this rule were the patients with acute urinary obstruction who were

impairment of renal function of obscure origin) and were cared for under the diagnosis of chronic nephritis or nephropathy of obscure origin. In several of the cases the diagnosis was suspect quiescent pyelonephritis, but since these cases did not fill the criteria of pyelonephritis they were assigned to this series. In three of the cases the patients had coexisting diabetes mellitus. Autopsy or laparotomy was performed in three of the 18 cases.

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curve had become more stable (about 30 minutes after injection) a time constant of 40 seconds

Comments

The renal detectors were calibrated daily and adjusted to register the same level of radioactivity with a standard source of Na 131 I. When a test dose of 0.4 μ Ci 131 I Hypaque/kg bodyweight was used a peak count rate of 10,000 cpm was obtained in normal subjects. The variation of the graphic recorder was found to be less than 2 per cent from the mean value.

To check that the detectors were placed over the kidneys during the measurements i.e. where the count was highest control measurements were made at the end of the examination by manual scanning of the back. This control gave good agreement with the marking of the kidneys and only in eight examinations were new renograms necessary on the following day with the use of positions found by scanning (cases I B H H M I S B H Z A Z N).

Exceptions to these principles with indication and X ray film were made in the series with suspect acute ureteral obstruction where the radioisotope test was performed before urographic examination in the acute stage of disease. When the patients in this series were acutely ill with severe pain and in a poor general condition the measurements over the kidney were made for only 15–20 minutes. On examination of this series manual scanning was done after the initial phase of the

curve (about 20–30 seconds after injection). Immediately after the highest count was found, the definitive measurement was continued. Also in this series the detectors were checked regarding their positions over the kidneys by control scanning after the renogram test and here too good agreement was also found with but few exceptions (cases A B C N E R N W) in which scanning was repeated during the test because the patients had moved during pain.

Control of detector equipment

The shielding effect of the renal collimator was examined in two types of model experiments. In the first experiment 10 μ Ci 131 I labelled Hypaque in a tube with a volume of 10 ml was used as a source of radiation. The tube was moved in the vertical and horizontal planes in a tank filled with water during registration of the radioactivity. In this examination the front of the collimator was 1 cm from the surface of the water (Fig. 16).

In the second experiment a phantom of the torso urinary tract in water was built up simulating the conditions prevailing at renography. The torso was represented by a plastic 10 litre tank. The kidneys were represented by two 250 ml flasks (B and C) each containing 150 ml of water. They were submerged in the torso with their mid points 8 cm from the posterior surface of the phantom and 12 cm apart. A third flask (D) with a volume of 250 ml was placed in the midline 12 cm below and 10 cm anterior to the

examined without any previous examination at the roentgen department

The approximate position of the kidneys was marked with indicators on the patient's back, after which a roentgenogram was taken of the kidneys with the patient sitting upright. With the aid of the position of the indicators relative to the outlines of the kidney on the roentgen film it was possible to outline the kidneys on the patient's back. When the roentgen film failed to outline the kidneys the examination was repeated with longer exposure until the outline was satisfactory. In all patients examined urographically before renography the position of the kidneys was estimated in order to check that the patient had not a pelvic kidney, and to compare the sizes of the two kidneys in excretion urograms and in films with the patient sitting upright.

The examination procedure of renography

The tests were carried out in the morning between 8 00 and 9 00 a.m. with the patient sitting comfortably during the test leaning slightly forward with a shielded scintillation detector placed against the chest. The detector was held in the front in the midline with the collimator close to the skin and the cranial edge of the outer opening at the manubrium sterni.

The two detectors over the kidneys were placed with their apertures in contact with the back of the patient. The detector units were directed

obliquely downwards (about 45°) and outwards (about 15°) from the midline (see Fig. 15).

Injection The dose of radioactive substance was injected intravenously into a cubital vein and, to avoid extravascular injection of the radioactive substance and at the same time standardize the injection technique blood was always aspirated in such an amount in the syringe (1 ml Mantoux syringe) that the latter was filled before the injection which was then given during a period of 5–10 seconds. The injection was never accompanied or followed by any complications.

Recording Recording of the chest and renal curves was continued for 45–60 minutes. During the first 10–15 minutes a time constant of 25 seconds was used and then a time constant of 10 seconds and after the



FIGURE 15. Arrangement for determination of the ^{131}I Iliopaque renogram.

Results and comments

The measurements showed that each detector measures also \approx per cent of the activity in the contralateral kidney and 6 per cent of the activity in the bladder. This means that every renogram is influenced to some extent by the activity in the contralateral kidney. In practice however this influence is negligible as is clear from the following example where the influence was maximal. In case L 3 (right renal aplasia) the counts for the calculation of uptake ratio on the right side were 4800 and 3900 cpm on the right side and 6400 and 8000 cpm on the left. The uptake ratio on the right side was 0.81. If the values are corrected for the 5 per cent of the simultaneous counts from the left side the ratio will be 0.78 instead i.e. an error of only 4 per cent. In all of the cases with a smaller difference between the two sides the error must therefore be still smaller.

During the first minutes after the injection the bladder activity was low and could therefore hardly influence the uptake ratio. It must however later disturb the excretion ratio but is least when excretion is impaired. The bladder activity and the body background probably help to explain why the excretion segment of the renogram falls slower towards the end of the test.

Roentgen examinations

The roentgen examinations were done at the roentgen diagnostic department

by routine methods (determination of the size of kidney, excretion urography, retrograde pyelography, selective renal angiography or aortography).

In the examination of the cases with acute ureteric obstruction where urography was done in the acute stage of the disease the patients were examined without preparation and compression. Neither was any compression applied in this examination of patients with hydronephrosis while compression was used in patients with parenchymatous renal disease. For the roentgen examinations 20 ml of 43 or 60 per cent Urografin (Schering) was used.

Other laboratory studies

Determination of serum creatinine (normal values 0.5–1.2 mg/100 ml) was made according to the method described by Haugen & Blegen (1952) and modified by Loken (1954).

In the determination of endogenous creatinine clearance the urine samples were collected during a $\frac{1}{2}$ hour period and determinations of the endogenous plasma creatinine were made. Only voided urine was used. Normal values 90–148 ml/min. The creatinine clearance is corrected to hold for 1.73 m² body surface area.

Determination of the serum N P N was done according to the method of Rippaport & Fiehlhorn (1947). Normal values 20–40 mg/100 ml.

The test of maximal specific gravity was done according to the method described by Addison & Shetky (1922).

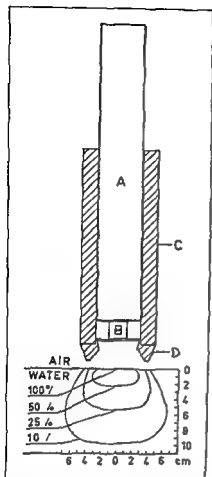


FIGURE 16 Scintillation counter used for renography and isoresponse curves in water A Scintillation detector B Crystal C Lead shield D Lead insert of collimating aperture

kidneys representing the urinary bladder (Fig 17) The radioactivity was measured with the two scintillation detectors whose collimators were in contact with the surface of the torso All counting rates were corrected for background The experiments were done both with and without radioactivity in the torso outside the flask In three experiments, 1 3 and 10 μCi radioactive Hypaque respectively was added to the water in the torso The right flask was at the same time given a series of increasing doses of radio

activity (1, 2, 3, 4, 6, 8, 10 μCi) while the left flask was not given any radioactive solution during the entire experiment The activity from the detector over the left flask was expressed in per cent of the activity from the right

In the experiment with the "bladder" neither the torso nor the kidneys contained any radioactive solution The radioactivity was determined while the bladder was receiving a series of increasing amounts of radioactive Hypaque (1, 2, 4, 6, 8, 10 μCi) The bladder was empty from the beginning of the experiment but was given 5 ml water together with every dose, and at the end of the examination it contained 30 ml

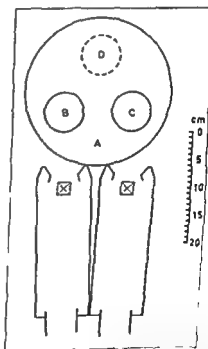


FIGURE 17 Phantom torso urinary tract used for study of shielding effect of the renal collimator A Plastic tank B and C Flasks representing the two kidneys D Flask representing the bladder

ney. On experimental ligation of the ureter the tracing showed a continued rise.

It is generally accepted that the second segment is an approximate measure of renal function and the third segment is related mainly to renal drainage. The opinion put forward by Taplin et al (1956) and Winter (1959) that the initial segment is a measure of vascular capacity has however been assailed by other investigators.

Wax & McDonald (1962) who used ^{131}I Hippuran examined the vascular capacity of the kidney and the background from the surrounding tissues in dogs and humans. The background was determined with radioactive albumin and measurements were made before and after nephrectomy. They found that at 1 minute \pm close to the time of the spike of the vascular segment only 16 per cent was contributed by vascular capacity and 59 per cent by the functional components. At the time for the maximum height of the curve the vascular capacity was only 5–10 per cent of total height at that time. The transfer system of ^{131}I Hippuran could be competitively inhibited by administration of sufficient doses of PAH. The effect caused a marked decrease in amplitude of both the initial rise and maximum height of the curve and also a slowing of the excretory segment by flattening of the curve. This led to the conclusion that a large fraction of the initial segment represented tubular uptake and that less than half of it reflected inflow of isotope containing blood. Klapproth et

al (1962) performed similar experiments on dogs and obtained the same results.

Wax & McDonald (1962) also showed that after administration of Probenecid the second segment of the curve was depressed as much as 70 per cent. There was an excellent correlation with the magnitude of this depression and the reduction of the clearance of PAH. Similar results by saturation with PAH have been reported by Montandon et al (1962) in rabbits and zum Winkel (1964) in rats with radioactive Hippuran and Hypaque renography.

Klapproth et al (1962) showed that osmotic diuresis increased urine flow sharply and depressed the amplitudes of the renogram. On decrease of the flow of tubular fluid by ureteric occlusion or by occlusion of the renal vein the curve rose continuously. Klapproth et al (1962) concluded that during the second segment of the curve which had been assumed to represent tubular accumulation of the isotope much of the isotope was accumulated not only in tubular cells but also in tubular fluids.

A number of investigations have been carried out on various animals to study the obstruction of the urinary pathways and its effect on the renogram especially on the third segment (O'Connor et al 1961, Klapproth et al 1962, Hretschmar & Greene 1963).

Taplin et al (1956) showed that ligation of the ureter resulted in a continuously rising curve as a sign of accumulation of the isotope in the kidney without any signs of excretion.

Lowest normal specific gravity 1 025
Urine volume < 400 ml

The values for endogenous creatinine clearance and serum creatinine or N P N and concentration tests were determined during the same hospital spell as the isotope test, but at an interval of 0—7 days between the examinations. The corresponding interval between renography and roentgenography was 1—14 days.

C Clinical evaluation of radioisotope renogram

Evaluation of the external renal curves has been the subject of much debate in recent years and several types of procedures have been described or suggested. The discrepancies between the results of these analyses have made it difficult to decide which parameters are most useful for clinical use. A brief summary of experimental data obtained in animals and in human beings therefore appears necessary for the evaluation and interpretation of the renogram segments and their changes under different experimental conditions.

Experiments in animals

Our fundamental knowledge of the different appearances of the renograms made under different experimental conditions has been described in various publications (DeMaria et al 1960, Denneberg et al 1960, Whitley et al 1961, zum Winkel 1961, Abbas et al 1962, Magnusson 1962, zum Winkel et al 1962, Beall et al 1963, Blau

fox et al 1963a, Wisenbaugh et al 1965).

zum Winkel (1964) gave a detailed description of a number of these experiments. He also reported personal experiments with ^{131}I Hippuran which were in part a further link of previously published experiments with various radioactive contrast media (Diodrast, Hypaque, Hippuran).

Tiplin et al (1956) discussed the different segments of the renogram on the basis of animal experiments. They thought that the initial segment of the curve (vascular segment) represented the vascular capacity plus that of the background tissue. When a detector was placed over the area where a kidney had been removed the spike of the initial segment was approximately one third of that obtained over a normal kidney. The secondary rise in the tracing beginning during the second or third minute and lasting three to five minutes (uptake segment) was due to tubular cell secretion of the contrast medium. Therefore the steepness and height of this segment was proportional to renal function. This segment was taken as a good index of total function of the kidney. When the kidney was absent or non-functioning or when the renal artery was blocked a secondary ascent did not occur. The second segment terminated at a maximum value after which the third segment (excretion segment) started 5—8 minutes after injection of the substance. There was a rapid fall for about 5—10 minutes and this fall represented elimination of the radioactive urine from the region of the kid-

accumulation of the radioactive substance in the kidney and intrarenal passage was shown in the experiments with ligation of the renal artery inhibition by Probenecid or saturation with unlabelled P³²H. These experiments (particularly in the ¹²⁵I Hippuran renogram) indicated that the second segment was closely correlated with renal blood flow (vascular capacity) as well as with the concentrating capacity of the kidney. This segment was affected also in its latter part by changes in renal drainage. The functional capacity of the kidney could thus be judged best from the second segment.

The second segment terminated at the maximum height of the curve. This proved to be dependent on variations in urine flow and changes in drainage e.g. by ureteric obstruction or venous occlusion. The amplitude of this maximum value of the curve increased when urine flow was low and the drainage impaired but decreased when it was high and the tubuli blocked.

From experiments with ligation of the ureter and low urine flow it was clear that the third segment was an expression for the elimination of the isotope from the region of the kidney i.e. a measure of evacuation from the field of vision of the detector. This segment elucidated mainly the disturbances in drainage. During this segment however uptake continued although elimination played a dominant role.

The results showed no essential difference between animals and human beings in the build up of the renogram.

The experimental studies may thus serve as a basis for interpretation and evaluation of the different segments of human renograms.

Experimental studies have shown that renograms are extremely complex. No direct measures of the components of renal function such as glomerular filtration, tubular secretion or renal blood flow could be recognised from the curves. On the other hand it was shown that the second and third segments were correlated with the accumulation of the test substance in the kidney and elimination of it from the region of the kidney. No distinct limits could be determined regarding the time of the start or end of uptake or excretion. The curve seems to 'reflect' a kinetic equilibrium of several aspects of the movement of renal fluids and of the tubular transport not interpretable in terms of specific renal functions (Klapproth et al 1963).

Technique of evaluation of renograms by other investigators

Investigations in animals and in man have shown that the renogram is a very complex curve and does not allow evaluation of glomerular filtration or tubular secretion separately. Attempts have however been made to obtain quantitative measures from the renogram. *Changes in one or more of the segments have been interpreted as signs of different types of disturbances of renal function.* The purpose of the present analysis was to find more objective criteria of normal and ab

Magnusson (1960) showed that the curve had a normal uptake segment, which meant that the capacity of the kidney to take up the isotope was intact. Denneberg et al (1961 b) showed that when the ureter was ligated for any length of time, pathological changes occurred and severely interfered with renal function. This was reflected particularly by the decreased uptake of ^{131}I Hypaque and by the retarded excretion. After eight weeks' ureteric ligation the kidney was probably not able to take up the test substance.

Studies in human beings

Investigations in man have been carried out under various experimental conditions to study the renogram and its various segments. In principle similar methods have been used as those in animal experiments, but modified for human beings (Winter & Triplin 1958, Merde & Shy 1961, Montandon et al 1962, DeMaria et al 1963, Hinc et al 1963).

As in the animal experiments, much interest has been focused on the evaluation of the initial segment as a measure of vascular capacity. Wax & McDonald (1962) carried out some of their investigations on patients under conditions described in the experimental part. This view that the initial segment reflects only part of the vascular capacity together with the non-renal vascular capacity and also tubular uptake was verified by Dore et al (1963) and Wedeen et al (1963).

Wax & McDonald (1964) demon-

strated a good correlation between the maximum height of the curve and PAH clearance in their clinical study. Dore et al (1963) found that different sized doses of PAH exceeding 100 mg progressively flattened the second segment. They compared the findings in this experiment with the results of measurements over the kidneys and the bladder (cystogram). They found that almost no tracer left the renal area during the second segment of the renogram. Since the tracer was retained in the kidney during this segment and since low plasma concentrations of ^{131}I Hippuran are almost completely removed, the rate of tracer accumulation or the slope of this segment of the ^{131}I -Hippuran renogram was an index of renal blood flow.

Conclusion of renogram experiments

The results obtained in the experimental investigations and reported above can be summarised as follows: measurements performed before and after nephrectomy, experiments with RIS-1 and injection of large doses of unlabelled PAH, showed that about one third of the first segment represented body background. The rest of this segment represented renal blood content and a considerable functional component which argued for accumulation in the kidney. These data showed that the first segment was much more complex and did not represent the vascular capacity of the kidney to the extent originally supposed.

That the second segment of the curve was mainly an expression of the

has been described by Taplin et al (1956) Winter (1956 1957 1959 1963) Tauxe et al (1962) Doug et al (1963) Pedersen et al (1964) and others

2 Comparison with group envelopes

With this method several normal curves are plotted and an envelope is drawn which includes all these curves. The actual curve is then compared with this normal field (Weade & Shy 1961)

3 Calculation of different types of ratios, slopes, tangents, angles from one or the other of the three segments of the curve

Common to these parameters is that they give a measure of the slope of selected parts of the curve. Applied to the second or third segment the parameters can for example be used as measures of uptake or excretion of the substance. An example of such an uptake ratio is that between the two amplitudes of the curve $a_{2 \text{ min}}/a_B$ (zum Winkel 1964). A corresponding measure calculated from the excretion segment is g the amplitude at 10 min (150 min) in relation to the amplitude at maximum count of the curve (a_{max}) was used by Wedeen et al (1963). A further variant is the amplitude 15 min after the maximum count (a_1) in relation to the amplitude at maximum count (a_{max}). The ratio is then multiplied by 100. Subtraction from 100 per cent gives the difference per cent of relative retention (Iarmelant III et al 1964).

The slopes can be expressed as an angle or as a tangent, i.e., a measure of the rate at which the substance is concentrated or excreted in the area studied. These values have been determined and expressed as an angle or tangent of the original curve and one of its segments and partly by measurement of the half time of the excretion or uptake segment. The latter determination has, as a rule, been used after replotting the data on semilogarithmic paper with extrapolation of the slope of the third segment to its intercept on the ordinate. At different intervals the uptake value measured is subtracted from the excretion slope and the differences replotted after which the half time value of the exponential line is determined (Block et al 1960 a Roth et al 1960 Witcofski et al 1961 Magnusson 1962 Becker et al 1963 Dawborn & Doyle 1963 Taplin et al 1963 Krogsgaard & Frus 1964).

4 Determination of parameters from the time axis of the curve

This method gives durations of the different segments. Examples are the time to maximum count (t_{max}) i.e., interval between injection and the time of maximum count or from the injection to 75 per cent of the maximum count (t_E) or 50 per cent of maximum value (t_G) etc. (Spencer et al 1961 Stewart & Hayne 1962 Brown et al 1963 Johnson & Odom 1964 Krogsgaard & Frus 1964).

A special method for analysis of the first segment has been described by

normal curves, preferably from parameters easy to calculate without complicated analysis of the curves

Taplin et al (1956) and Winter (1956, 1957, 1959) thought that renography should be regarded simply as a qualitative test allowing no quantitative analysis. In the evaluation of the renogram they considered the general shape of the curve and regarded the first segment as an indicator of vascular capacity. But as mentioned in the experimental investigations, the first segment proved a poor measure of vascular capacity because of the considerable extrarenal and functional components. The initial segment has proved less suitable for the evaluation of vascular capacity in clinical practice, too (Stewart & Haynie 1962, Dawborn & Doyle 1963, Farmelant et al 1964).

Most investigators have also preferred to calculate their parameters from the second or third segment of the curve. Different types of parameters have been chosen and applied to subjects with and without renal disease and the results have been compared with the clinical findings renal function data and different types of roentgen examinations. The examiners have selected one or usually a combination of several parameters to characterise their curves.

The methods used are briefly outlined below and Fig 18 shows reference points used by various investigators in the analysis of the renogram. Some investigators have defined the O point (t_0) from the beginning of the injection, others at the end of the

injection, but also at the time when the initial segment of the curve rises above background activity. In the figure point O is set at the end of the injection.

1 Visual comparison of the curves

This method compares the curves regarding (a) the changes in the three segments, which occur in various renal diseases and in different degrees of impairment of renal function (b) the amplitudes of the curve at different times. The amplitudes measured were the maximum value of the first segment (a_B), the height at maximum count (a_{max}) and the height at some selected time of the excretion segment, e.g. 10, 15, 20 or 30 minutes ($a_{10 \text{ min}}$, $a_{15 \text{ min}}$, $a_{20 \text{ min}}$, $a_{30 \text{ min}}$). Evaluation according to these principles

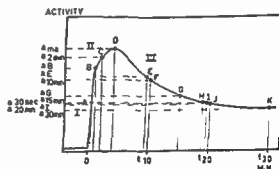


FIGURE 18 Reference points used by various investigators in the analysis of the renogram. O Time of injection. I 1st segment. II 2nd segment. III 3rd segment. Amplitudes: A 30 seconds after injection. B of 1st segment. C 2 minutes after injection. D at maximum height. E 75 per cent of maximum height. F 10 minutes after injection. G 15 minutes after injection. H 50 per cent of maximum height. I 15 minutes after maximum height. J 20 minutes after injection. K 30 minutes after injection.

procedure with amplitudes and used parameters of the types described under points 3 and 4 instead Brown et al (1963) and Kroghgaard & Ivers (1964) used t_{max} respectively 50 per cent of maximum value (t_c) while a number of other examiners used t_{max} in combination with one or more other parameters to describe the renogram (Roth et al 1960 Merde & Sky 1961 Boyd & Murdoch 1962 Weden et al 1963 Johnson & Odum 1964 Scholtz et al 1964 zum Winkel 1964 and others)

In a few large clinical series the investigators have compared their own evaluation technique with those used by others Stewart & Haynie (1962) reported that the height of the first segment (a_B) was poorly correlated with renal artery stenosis as well as the maximum height of the curve (a_{max}) and the value 15 minutes after injection ($a_{15 \text{ min}}$). They compared t_{max} and t_c with their clinical results but also these parameters relatively often give false negative renograms (23%) as well as false positive renograms (27%). When they used γ_{max} as the only parameter 45 per cent proved false negative and 47 per cent false positive. According to Farmelant et al (1964) and Wax & McDonald (1964) this high percentage of false negative and positive renograms in Stewart and Haynie's (1962) series was due to their not having compared one kidney with the other of the same patient but with normal control values.

Farmelant et al (1964) analysed renograms with the retention quotient given under point 3 which they used as an index between the kidneys. They

found a difference of 20 per cent or more in this retention quotient in patients with known renal arterial stenosis. They compared their parameters with t_{max} to the quotient between the amplitude of maximum height and the ratio between the activity on each side after half a minute (130 sec). The use of t_{max} gave correct results in 55 per cent to 83 per cent γ_B in 72 per cent and $a_{30 \text{ sec}}$ in 56 per cent.

Wax & McDonald (1964) used four parameters (t_{max}) maximum height of the curve (a_{max}) the ratio between 15 min value of the curve and the maximum height ($a_{15 \text{ min}}$) and the angle to maximum height of the curve (α) the angle between the second segment and the base line as measured with a protractor. To evaluate any disparity the left value was compared with the right one. This evaluation method gave 24 per cent false positive and 7 per cent false negative. When they used the criteria of Farmelant et al (1964) 10 per cent were false positive and 48 per cent false negative. Wax & McDonald (1964) recommended the use of the renogram as a screening test to eliminate the majority of hypertensive patients in whom renovascular disease was not the cause of their hypertension. With their method they detected 93 per cent of patients with renovascular hypertension.

Other investigators have reported fairly good results with the use of an index between the kidneys or differences between the kidneys for evaluation of disparity but the series were

Witcowski et al (1961) They used a technique with a short time constant and a rapid recorder and used three time intervals during the initial segment. This analysis of the first part of the curve was combined with determination of half-times of the second and the third segments, according to the principles given above.

5 Other methods used

A number of methods involving more complicated analytical procedures have also been used. Thus, Paker et al (1960) plotted the ^{131}I Diodrast renogram curves on arithmetic paper and evaluated the heights of, and areas under, the three segments of the curve. Krueger et al (1961) used a method, according to which the amplitudes were determined at the end of the first segment (a_B) and the amplitude of the maximum height (a_{\max}), after which $100(a_{\max} - a_B)$ was determined as a measure of the total concentration. The total concentration was divided by time during the first segment of the curve and the time during which the curve returned to a value equal to the height of the first segment. The minute concentration respectively minute excretion were calculated from these data.

Hirakawa & Corcoran (1963) proposed a formula based on measurements of the first segment (a_B), the height of the second segment (a_{\max}) and the 15 minute level ($a_{15 \text{ min}}$) after which they used a formula

$$\frac{(a_{\max} - a_B)^2 + (a_{\max} - a_{15 \text{ min}})^2}{a_{\max}^2}$$

Attempts have also been made to use a more theoretical analytical evaluation of the 'true renal curve' by subtraction of the blood background curves (Horst et al 1961, Magnusson 1962, Tauxe et al 1962), and more extensive mathematical analysis, compartment analysis (Blumsohn et al 1963 c, Spencer & Sigman 1963, Coe & Burke 1964, Kulka et al 1964).

Spencer et al (1961) reported a systematic analysis of various parameters calculated from the ^{131}I Diodrast renogram. They found certain slopes and ratios of amplitudes to give the best reproducibility. Their results showed that calculations made simply on the basis of amplitudes of the curve were difficult to control *inter alia* because of variation of the detector and kidney geometry.

Pircher et al (1963) also found that individual differences in thickness of the absorbing tissue and the distance between the detector and the kidney made it impossible to use amplitudes. Despite these objections Pircher et al (1963) recommended maximum height count (a_{\max}) the count 15 minutes after injection ($a_{15 \text{ min}}$) and a_{\max} which they found superior in the analysis of 14 parameters and difference of the parameters between the left and the right kidney. Clinically however, several groups have used amplitudes and reported good agreement between such values and their clinical data (Tauxe et al 1962, Burbink et al 1963, Doig et al 1963, Pedersen et al 1961).

A number of other groups have however questioned the value of this

may be measures of the same function. Two parameters calculated from the second and third segment might be sufficient to evaluate the renal curve. Parameters such as ratios calculated from the second segment for the evaluation of uptake function or from the third segment for estimating elimination of the substance would probably be the most suitable for clinical work.

Choice of parameters of the ^{131}I -Hypaque renogram

Fig. 19 a shows an original ^{131}I Hypaque renogram. The renogram shows a rapid initial rise of activity 10–20 seconds after the injection of radioactive Hypaque. This initial upswing is followed by a further ascending segment which rises less rapidly than before to reach a maximum value at 2–6 min after the injection. This is followed by a descending segment with a gradually decreasing disappearance rate from the maximum value to about 20–30 min. During the rest of the examination the activity gradually disappeared at a constant rate. The height of the initial rapid upswing is often difficult to determine with precision because of its continuity with the next segment.

Fig. 20 gives a schematic diagram of the normal ^{131}I Hypaque renogram showing the time of injection and the time at which the two parameters are calculated. As a measure of uptake the ratio between the value at 35 min and that at 15 min were calculated from the second segment of the curve.

The ratio between the value at 15 min and at 30 min after the injection was used as a measure of the elimination of the substance (excretion ratio). It is important that these two points are not close to maximum value, but they should not be too late in the curve. The choice of an early value of the curve involves the risk of an increased effect of uptake function which is still going on while a late value increases the risks of an effect of the activity from the bladder. The graphical description of the renogram is done in an arithmetic scale and all the values are corrected for background activity.

In the acute urinary obstruction series (series II table XVIII) the excretion ratio (II) was calculated as the ratio between the 3 minute value and the 15 minute value because in these cases most of the curves could only be recorded for a short period.

Fig. 19 b shows an original external blood curve measured over the chest. The values at 5 and 30 minutes were used to form a ratio which were multiplied by 100 to give a measure of the retention per cent of the isotope.

Theoretical analysis of the renogram and the parameters used

In the analysis of the different factors forming an integral part of the complex external renal curve reference is made to a schematic model of the passage of the test substance through the kidney (Fig. 21). The detector over the kidney records the net effect of simultaneous processes: i.e., the arrival of

smaller than those referred to above (Doig et al 1963, Hirakawa & Corcoran 1963, Wedeen et al 1963, Krogsgaard & Frus 1964, Pedersen et al 1964)

Summing up, though the above mentioned investigators reported varying results of their evaluation procedures, it is generally recognised that the renogram should be evaluated by standard parameters. Simple parameters calculated from the second or third segment have been used in the evaluation of the individual curves and for demonstrating disparity between the two kidneys. More complicated procedures have also been tried for recognising any difference between the two kidneys in a given patient, but such methods are laborious and are therefore not really satisfactory for clinical use.

The Author's method for evaluation of ¹³¹I-Hypaque renogram

Evaluation of renograms by visual inspection and from the value of t_{\max} has proved less satisfactory (Denneberg & Hedenskog 1959).

Visual inspection is however, sufficient to demonstrate or exclude urinary obstruction (Denneberg 1959/60). t_{\max} in combination with a ratio between the maximum value and the value 30 minutes later implied a considerable improvement (Denneberg 1962). This procedure was not really satisfactory either because it did not allow any definite estimation of uptake function. In studies on cats with

experimental hydronephrosis ratios were calculated from the 2 and 24 minute values of the renogram as a measure of renal uptake (Denneberg et al 1961b). The purpose of this ratio was to facilitate comparison between the different curves after various intervals of unilateral ureteric occlusion and to make it possible to follow the course of the impaired renal function.

Experimental studies and clinical experiences have shown that the first segment of the renogram is less suitable for the calculation of the parameters, it is too complex and does not properly reflect the vascular capacity of the kidney. Another drawback is that the vascular capacity is estimated from a single amplitude and not from a ratio. Such amplitudes proved unsuitable because of their dependence on the radioactive dose and sources of error due to the kidney detector geometry. In the interpretation of the renogram it was desired to obtain parameters which were independent of the above mentioned factors and which could be calculated for example as a ratio between two amplitudes. These ratios should be simple and easy to calculate and it should be possible to use their primary values in the original curve. The information obtainable from such parameters should also give a satisfactory description of the changes in the curve due to changes in renal function. Thus the information obtainable from the curve does not vary with the number of parameters used. As exemplified above (point 3 page 63) several parameters

reaches its maximum there is equilibrium between inflow and outflow. When the outflow i.e. elimination of the substance is predominant the curve falls. It should however be underlined that the latter term is to be understood as elimination from the field of vision of the crystal and is not a measure of excretion from the renal pelvis.

ACTIVITY

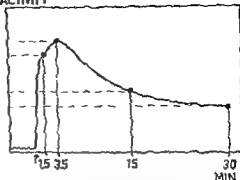


FIGURE 20 Reference points used in the analysis of ¹³¹I hippurate renogram in the present investigation. Arrow: Time of injection.

With the aid of two parameters calculated as ratios from the rising and falling segments of the curve it is possible to obtain four combinations of theoretical kidney curves corresponding up to different types of disturbed function. These curves are exemplified in Fig. 22. Type A has a normal rate of inflow and outflow while B represents a curve with decreased rate of inflow but normal outflow. This curve shows decreased uptake capacity with an abnormally slow rise which causes a delay of the maximum value of the curve. The excretory segment shows a slower decline because uptake of the substance persists longer than normal. This means that a ratio calculated from this segment decreases with impairment of uptake function even if outflow is not obstructed. Type C shows a normal inflow but decreased outflow while type D shows both decreased inflow and outflow.

Fig. 23 gives a theoretical survey of the expected relation between uptake and excretion ratio. The figure shows how the two ratios should be for the four different types of curves which are given with their numbers in separate fields. Type A is located in the field with normal uptake and excre-

tion ratio while type B shows (depending on the severity of the impairment of uptake) a successive movement from the upper right field to the lower left on the diagram along the broken line.

That none of the ratio values are within the lower right field is explained by the fact discussed above that despite free drainage the excretion ratio is decreased in kidneys with impaired uptake capacity. The example shows the close dependence of excretion ratio on uptake ratio from which it cannot be judged separately. Type B as a final stage lies lowest in the

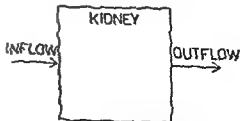


FIGURE 21 Schematic model of passage of test substance through the kidney.

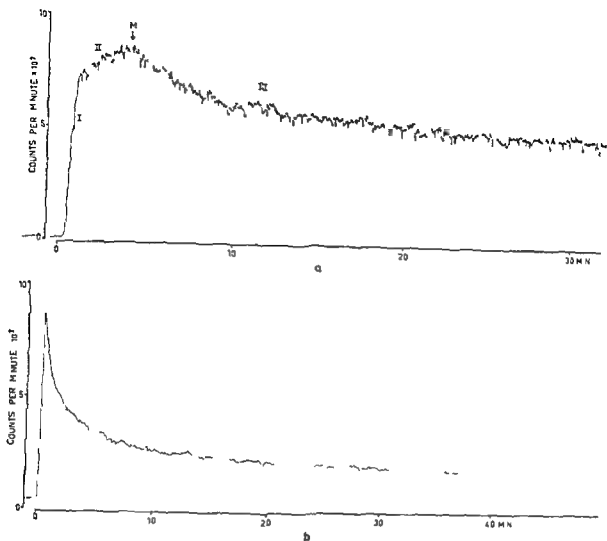


FIGURE 19 a Original ^{125}I Ilypaque renogram in a control O Time of injection I 1st segment II 2nd segment III 3rd segment M Maximum height b Original Ilypaque external chest curve

the substance and its accumulation in the kidney (inflow) and its elimination from the kidney (outflow). Inflow thus largely represents the renal circulation of the substance, its glomerular filtration and passage via peritubular capillaries into the tubular cells and out to the lumina of the tubuli, i.e., all processes contributing to accumulation of the substance in the kidney. As long as inflow is predominant, the curve will rise. The

other processes during the further intrarenal passage through the various parts of the nephron (convoluted and collecting ducts) down to the renal pelvis and the simultaneous reabsorption of water on the other hand produce no increase in the activity and therefore no further rise of the curve. The detector cannot distinguish these simultaneous kinetic processes but records only the sum of renal and extrarenal activity. When the curve

reaches its maximum there is equilibrium between inflow and outflow. When the outflow i.e. elimination of the substance is predominant, the curve falls. It should however be underlined that the latter term is to be understood as elimination from the field of vision of the crystal and is not a measure of excretion from the renal pelvis.

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ACTIVITY

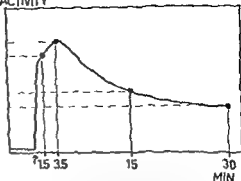


FIGURE 20 Reference points used in the analysis of ^{131}I Hypaque renogram in the present investigation. Arrow: Time of injection

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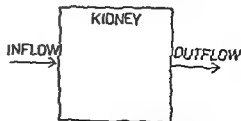


FIGURE 21 Schematic model of passage of test substance through the kidney

% OF DOSE

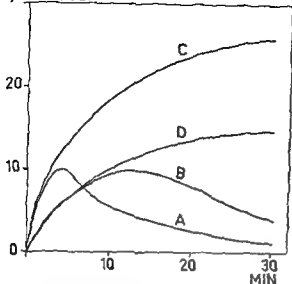


FIGURE 22 Theoretical kidney curves for different types of disturbed function (A, B, C and D see page 69)

left field, and the curve is then identical with a body background curve.

In principle one might imagine a type of curve localised in the field to the right above the normal field (marked with broken line and arrow). This curve represents abnormally high uptake with a normal excretion ratio and might correspond to a hypertrophic kidney. Type C is localised in the upper left field and shows decreased excretion ratio with normal uptake. This would correspond to the type of curve seen in acute urinary obstruction. On removal of the obstruction it would return to the normal field. The last type (D) corresponds to impaired uptake (decreased uptake ratio) in combination with decreased excretion. This curve moves towards the lower left field where the uptake has ceased and corresponds to a non-functioning kidney in the final stage.

It should be stressed that these types of curves are based on theoretical renal curves and not on true renograms.

One must also consider factors outside the kidney, e.g. effect of extrarenal activity (Fig. 24). The figure shows the time curves of the distribution of ^{131}I Hippuric in the organism after an intravenous injection. P represents the blood activity, P + E the sum of the activity in the blood and extravascular space and U the cumulative excretion to the bladder. During the ascent and descent of the kidney curves, curve P shows a successive fall in blood activity and curve U, after an initial delay, rises as a sign of continued accumulation of radioactivity in the bladder. A substance with high clearance rate e.g. ^{131}I Hippuran, has a steeper second segment and the dec-

UPTAKE

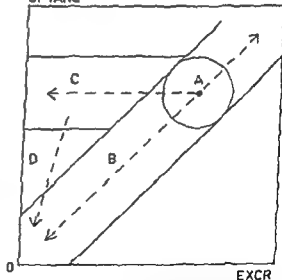


FIGURE 23 Theoretical survey of expected relation between uptake and excretion ratio. A Normal function B Decreased rate of inflow C Decreased rate of outflow D Decreased rate of inflow and outflow

line of the third segment is more marked than for substances with lower clearance rate as $g^{231}\text{I}$ Hypaque. This is reflected also in curve P by a more rapid decline in the concentration and in curve U by a more rapid rise of the bladder curve.

The effect of the non renal factors on the renogram can thus complicate the use of the parameters. Despite this objection it is of interest to ascertain whether parameters can be calculated from the more complex renogram seen in different types of renal impairment to test the schematic renal model, and to find out to what extent theoretical analysis of kidney curves agrees with the renograms used in clinical practice. The results of the renograms can be illustrated by a similar diagram with the two parameters plotted against each other.

Fig. 23 a and b are based on 173 and 182 kidneys from series A—I for comparison with different types of functional disturbances. An account is given of four respectively five groups including normal kidneys, kidneys with known urinary obstruction (acute or chronic), kidneys from patients with other renal diseases without known urinary obstruction and finally a group with aplasia status post nephrectomy or a silent kidney. Verification of the presence or absence of urinary obstruction was obtained by urography and/or retrograde pyelography, renal manometry or post mortem examination.

It is clear from Fig. 23 a and b that the distribution of the various function groups among separate fields

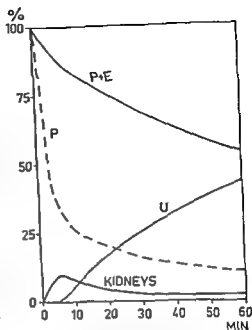


FIGURE 21 Schematic survey of fate of I^{131}I Hypaque after single injection P Plasma curve P + E Sum of plasma and extra vascular tissue U Cumulative urinary excretion.

was largely the same as that in the theoretical diagram (Fig. 23). The borders of the fields of these groups were based on observed values. Fields with normal uptake and excretion ratio occupied corresponding positions (I and A) and thus holds also for the fields with decreased uptake and normal excretion ratio (II and B). The two fields C and D in the theoretical diagram on the other hand show only one field (III) in which the series with verified urinary obstruction (acute or chronic) are localised. In Fig. 23 the upper right part contains a field above the normal field (IV). The former includes cases with an abnormally high

% OF DOSE

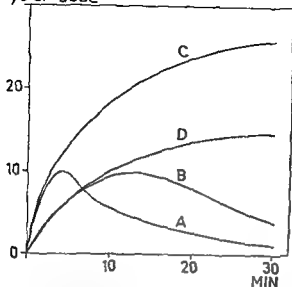


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In principle one might imagine a type of curve localised in the field to the right above the normal field (marked with broken line and arrow). This curve represents abnormally high uptake with a normal excretion ratio and might correspond to a hypertrophic kidney. Type C is localised in the upper left field and shows decreased excretion ratio with normal uptake ratio. This would correspond to the type of curve seen in acute urinary obstruction. On removal of the obstruction it would return to the normal field. The last type (D) corresponds to impaired uptake (decreased uptake ratio) in combination with decreased excretion. This curve moves towards the lower left field where the uptake has ceased and corresponds to a non-functioning kidney in the final stage.

It should be stressed that these types of curves are based on theoretical renal curves and not on true renograms.

One must also consider factors outside the kidney, e.g. effect of extra renal activity (Fig. 24). The figure shows the time curves of the distribution of ^{131}I -Hypaque in the organism after an intravenous injection. P represents the blood activity, P + E the sum of the activity in the blood and extravascular space and U the cumulative excretion to the bladder. During the ascent and descent of the kidney curves, curve P shows a successive fall in blood activity and curve U, after an initial delay, rises as a sign of continued accumulation of radioactivity in the bladder. A substance with high clearance rate, e.g. ^{131}I Hippuric acid, has a steeper second segment and the dec-

UPTAKE

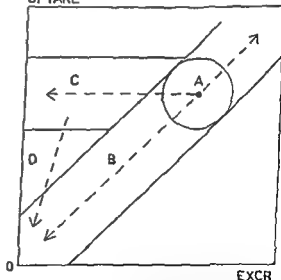


FIGURE 23 Theoretical survey of expected relation between uptake and excretion ratio. A Normal function B Decreased rate of inflow C Decreased rate of outflow D Decreased rate of inflow and outflow

GB (acute ureteral obstruction) shows the curve seen in urinary obstruction with normal uptake but decreased excretion ratio Type IV (case I A) illustrates the type seen for renal hypertrophy with abnormal large uptake and with normal excretion ratio

D Results

Controls (Series A)

Table XX gives the patients initials sex age the three renogram parameters (uptake excretion ratios I and II) of the kidneys retention per cent calculated from the chest curve N P A and the diagnosis Statistical analysis with the Student's *t* test showed that the differences between the respective parameters (right minus left) did not differ significantly from zero ($P > 0.05$) i.e. there was no significant difference between the sides compared renal uptake or the two excretion ratios The normal limits were therefore determined for the pooled series irrespective of the side Owing to the skewed distribution of uptake respectively excretion ratio I the mean value (M) and the standard deviation (SD) were determined from the logarithmic values of the series In the determination of the corresponding values for excretion ratio II the original values were used because the distribution of this material was normal Since no significant difference was found between the two sides the two renograms were directly comparable with one

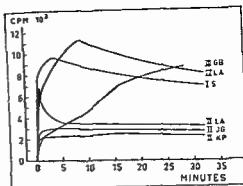


FIGURE 26 Various types of ^{131}I Hypaque renograms in 7 cases. The Roman numbers indicate fields given in Fig. 20 a Case B C (Series A) Control (I) Case A P (Series D) Congenital multiple cystic kidney (II) Case J G (Series E) Nephrosclerosis (II) Case L A (Series G) Right renal aplasia (II) and left kidney enlarged (compens. hypertrophy) (IV) Case G B (Series H) Acute ureteral obstruction. (III)

another and any difference between them may be ascribed to differences in renal function

Criteria of normal ^{131}I Hypaque renogram

A Hypaque renogram was accepted as normal when the values of both parameters were within the normal limits of the control series The mean uptake ratio was 1.075 and $M \pm 2 \text{ SD } 1.017-1.131$ Corresponding values for the excretion ratio I were 1.194 with a range of 1.085-1.316 and for excretion ratio II 1.268 respectively 1.087-1.490

The normal range of the excretion ratio II was wider than that of excretion ratio I This is because excretion ratio II is a less true measure of elimination because it measures a considerable component of the uptake

uptake ratio, u , the type of curve seen in renal hypertrophy, which is dwelt on in the chapter on the results (chapter VIII D). The agreement found between the parameters calculated from the theoretical curves and the more complicated renograms show that the method of evaluation can be used in clinical practice.

Fig. 26 shows different types of renograms from the clinical series, which correspond to the figures given in the fields. Type I (case S G) is a

normal curve. Type II includes three variants of curves representing different degrees of impaired uptake function. The uppermost curve does not show any uptake segment, but only a slowly falling excretion segment, i.e. the type seen in non-functioning kidneys (case L 1, renal aplasia). The other two curves show decreased uptake ratio, which influences the excretion ratio and also causes a delay of the maximum value of the curve (cases J G and K P). Type III (case

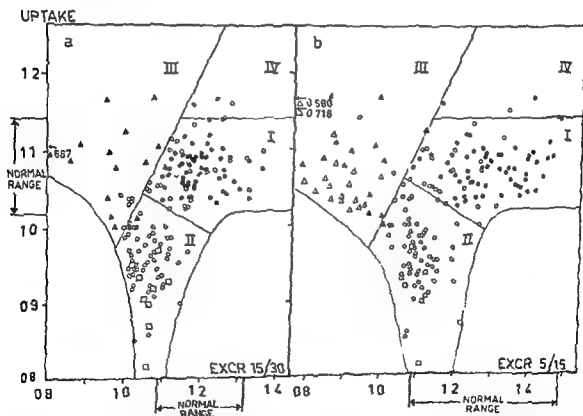


FIGURE 26a Uptake ratio plotted against excretion ratio 15/30 (I) in different types of functional disorders in 173 kidneys of series I—I except series D and H. Demarcation lines of the fields (I—IV) based on observed values. Solid circles: Control kidneys. Open circles: kidneys with various diseases without signs of obstruction. Solid triangles: kidneys with signs of chronic obstruction. Open squares: aplasia post nephrectomy or silent kidneys. b) Uptake ratio plotted against excretion ratio 5/15 (II) in different types of functional disorders in 182 kidneys of series I—I except series D. Open triangles: kidneys with signs of acute ureteral obstruction. Other symbols as in fig. 26a.

function with a consequently wider scatter of this parameter. As discussed in Chapter VIII B, however, the choice of excretion ratio II was dictated by the conditions prevailing at the time of the examination.

Disparity between the two external renograms was said to exist when the differences between the respective parameters (right minus left) were larger than those observed in the control group (Fig 29). In Fig 29 the difference is given irrespective of sign.

Reproducibility

The reproducibility of the method was checked by determinations at two days interval in ten controls. The results (given in Table XVI and Fig 27 b) showed no significant difference between the parameters calculated from the renograms (uptake and excretion ratio I). The error of the method (S.E.) of the uptake ratio was found to be 2.7 per cent, while the corresponding error for the excretion ratio I was 2.7 per cent.

Renal disease series (B—I): Analysis of uptake and excretion ratio

Tables XVII—XX show the cases in the various series of renal diseases with regard to initials, sex, age, the three renogram parameters (uptake and excretion ratios I and/or II) of the kidneys, retention per cent calculated from the chest curve and laboratory findings (N.P.N., serum creatinine, creatinine clearance, maximal specific gravity) and diagnoses. The cases in the series with paren-

chymal renal disease including hypertension, are arranged according to the severity of impairment of renal function. The acute obstruction series is divided into two groups according to whether the diagnosis was roentgenologically verified or not.

Figs 27 a—t and 28 a—c give the uptake by each kidney and the excretion ratio (I respectively II) in the control series and in the various series of renal diseases. Five cases were not included in the series for technical reasons (V B, L L, K J, K K and A B). The limits of the four fields discussed above (page 71) are inserted in each figure. To compare the values for the two sides in individual patients the two values have been joined.

Field I in Fig 27 a—t shows the values for controls and for patients with normal values in the other series. The latter represent half of the cases in the series with hypertension and other parenchymal disease while the majority of the remainder in these series fall within field II. Field II comprises all the cases with chronic glomerulonephritis and the majority of kidneys in the series of chronic pyelonephritis and polycystic kidneys and in the lower part of the field cases with aplasia and status post nephrectomy or silent kidneys. The poorer the uptake the lower the position of the ratio in the field. There is good agreement between the position due to changes in uptake ratio of the individual kidneys and function as judged from creatinine clearance, serum creatinine and N.P.N. It is noteworthy that the series of aplasia or status post

horizontal difference was found between the sides and as mentioned above the pathological obstructed side fell within field III (Fig. 27). The contralateral kidney fell within the normal field or sometimes in field II. This field also comprises three of the patients with hydronephrosis where the contralateral kidney was functionless or had been removed.

Figs. 28 b and c where the uptake ratio is compared with the excretion ratio 5/15 (II) show the same distribution of kidneys with obstruction as that of kidneys with hydronephrosis in Fig. 27. The majority of the contralateral kidneys in the patients with unilateral urinary obstruction fell within the normal field. The remaining cases in this series with non-verified urinary obstruction showed small differences and the majority of them were situated within fields I or II. One exception in this latter series was case M J who had undergone nephrectomy and in whom the remaining kidney was normal which explains the considerable difference between the sides.

Fig. 29 shows the difference between the right and left kidneys regarding uptake and excretion ratios I and II in all series (A—I). Nine of the cases in the series parenchymal renal disease including hypertension showed abnormally large differences. Eight of the cases differed in uptake ratio while five of the nine also differed in excretion ratio. Of these nine cases three belonged to series C (cases B M, B Z, V I); two to series D (cases K P, Z N); two to series E (cases H I,

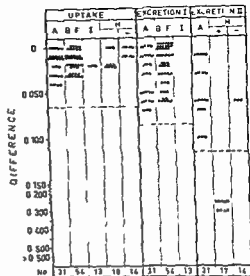


FIGURE 29 Difference between right and left renogram parameters in series A—I. Series II is divided into two groups with (+) and without signs of obstruction (—). 2 cases excluded for technical reasons (Series II).

K M) and two to series I (cases B F, I J).

In series II the difference in excretion ratio II clearly distinguished the roentgenographically verified cases with obstruction from those where roentgen examination revealed no obstruction. Only case C N fell within the limits of the control series and that patient was one of those who could not sit still during the test because of pain (C N, A B, E H and N W). In case A B the renogram was less satisfactory because after eight minutes recording the patient vomited and had severe pain and then moved from the detector over the left kidney. The excretion ratio (2/15) could therefore not be calculated. The patient was therefore excluded in the calculation

nephrectomy includes cases where the ratio for the contralateral kidney is situated in field IV. In these cases the roentgen examination verified compensatory hypertrophy of the kidney. Though the number of these cases is small, this localisation appears to confirm the validity of the previously described theoretical analysis. Field III in Figs 27a and 28b comprises the cases with urinary obstruction, i.e. with normal uptake but decreased excretion ratio. The majority of the contralateral kidneys in these series with out obstruction lay within field I or II, as did cases in the unverified cases of acute obstruction (Fig 28c).

Disparity between left and right renograms

As shown in Fig 27c-h the series with parenchymal renal diseases including hypertension and aplasia or

status post nephrectomy differed mainly along the vertical axis.

In the series chronic glomerulonephritis there was only a small difference between the values compared, while in the series chronic pyelonephritis, polycystic kidneys, other parenchymal disease and the series with hypertension the disparity was occasionally considerable. In these cases with disparity between the sides the "healthy" side usually fell within the normal field I while the affected side was situated in field II. Extreme differences were, of course noted in the series aplasia and status post nephrectomy, where the remaining kidney fell within the normal field or the field for hypernormal uptake ratio. The value over the site of the removed kidney fell within the lower part of field II, i.e. a sign of no uptake.

In the series with obstruction 1

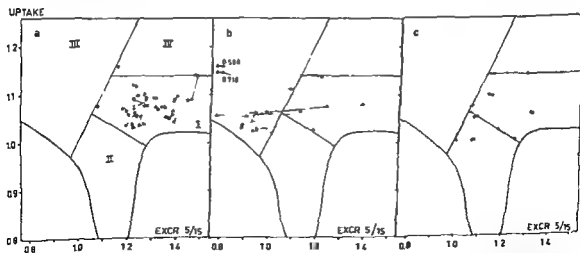


FIGURE 28 Uptake ratio plotted against excretion ratio (II) in series I and II. Ratio values in individual cases joined by lines. The Roman numbers indicate fields given in Fig 25b.

a Controls (Series I) b Cases with signs of obstruction c. Cases without signs of obstruction (Series II). One case in series II excluded for technical reasons.

horizontal difference was found between the sides, and as mentioned above the pathological obstructed side fell within field III (Fig. 27). The contralateral kidney fell within the normal field or sometimes in field II. This field also comprises three of the patients with hydronephrosis where the contralateral kidney was functionless or had been removed.

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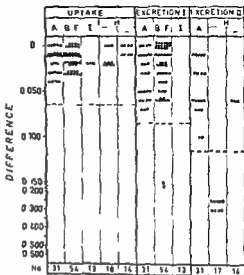


FIGURE 23 Difference between right and left renogram parameters in series A-J. Series II is divided into two groups with (+) and without signs of obstruction (-). 2 cases excluded for technical reasons (Series II).

K M) and two to series I (cases B J I J).

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In the series with obstruction, i

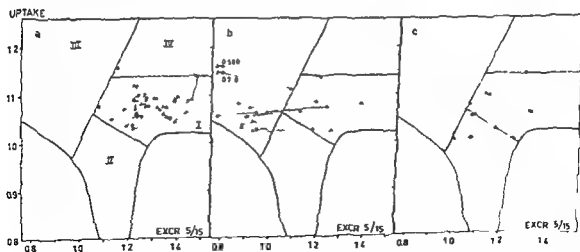


FIGURE 25 Uptake ratio plotted against excretion ratio $x/15$ (II) in series I and II. Ratio values in individual cases joined by lines. The Roman numbers indicate fields given in Fig 25b.

a Controls (Series I) b Cases with signs of obstruction c Cases without signs of obstruction (Series II). One case in series II excluded for technical reasons.

Table V II Comparison between urograms and renograms in cases of renal parenchymal diseases and hypertension (Series B—F)

Excretion urography Findings	Renography		
	Normal on both sides	Disparity between sides ¹	Both sides abnormal (no disparity)
Normal	3	0	7
Changes on one side	3	8	1
Changes on both sides	1	0	10

¹ Unilateral changes in all cases

Table V III Comparison between findings made at roentgen examination laparotomy autopsy and renography (Series B—F)

Roentgenography Findings	Renography		
	Normal on both sides	Disparity between sides ¹	Both sides abnormal (no disparity)
Normal	3	0	5
Changes on one side	3	8	1
Changes on both sides	1	1	22

¹ Unilateral changes in 8 and bilateral in 1

aminations and laparotomy provided the possibility of judging the morphological changes including differences in kidney size. While the roentgen examination and autopsy thus allowed estimation from a qualitative point of view, the renogram allowed evaluation from a quantitative functional point of view. The roentgenographic and autopsy findings were divided into three groups: (1) normal, (2) changes on one side and (3) changes on both sides. The renograms were classified as (1) normal on both sides, (2) disparity between the sides (unilateral or

bilateral abnormality) and (3) both sides abnormal (no disparity).

33 patients were examined with urography and renography (Table VII) and in 21 the findings made by both examinations were in good agreement. Corresponding figures in Table VIII show 44 cases examined of which agreement was found in 33. To elucidate the cause of the difference between the urography and renography, the findings were compared with the results of renal function tests (creatinine clearance, serum creatinine, N P N).

of the difference in excretion ratio in Fig 29, but is given as a roentgenographically verified case of obstruction in the other diagrams for the right side because the curve for this side ascended continuously without showing any maximum value during the measuring time

The difference in uptake ratio on the two sides for the corresponding series showed that cases E R, A B and N W fell outside the limits of the control series as well as case M J with aplasia (not marked in Fig 29) The values for the remaining 15 cases with, and 14 without, roentgenographically verified obstruction fell within the limits of the control series

In three of the thirteen roentgenologically verified cases of hydronephrosis in series I the difference in excretion ratio 15/30 fell within the limits of those of the control series One (S A) of the cases, however, had roentgenographically verified bilateral hydronephrosis Cases E H and I W had unilateral pathological curves on the roentgenographically verified pathological side But the difference between the two sides lay within the limits of the control series In five of the cases the difference in uptake ratio was abnormally large In one case the difference could be explained by a tumour of the pelvis which had caused extensive destruction of the renal parenchyma (case I W) In another case (S A) with bilateral hydronephrosis, it had proved necessary to reimplant the left ureter after the kidney had been shut off for a long time following an operation because of

gynaecological cancer In the remaining cases the difference between the sides could be explained by angiographically verified silent kidneys (cases E H and F J) and in the third (I N) the patient had undergone nephrectomy

Comparison between findings made at excretion urography, laparotomy or post mortem examination and renography

In four cases (V B, L L, K J, k k) no comparison was made between the sides because of technical errors of the renograms on one side

The number of roentgen examinations in each series of renal disease (B—I) is given in Table VI Table XX summarises the results of the roentgen examination and post mortem examination together with the findings at laparotomy

Table VII compares the results of excretion urography and radioisotope renography and Table VIII the findings made at roentgenography, post mortem examination and laparotomy with those at renography The cases in Tables VII and VIII consist of series B—I, i.e. parenchymal renal diseases including hypertension The urograms were judged with respect to excretion time of contrast medium density of contrast medium morphological changes and significant differences in size between the two kidneys In some cases excretion urography was supplemented by retrograde pyelography selected renal angiography or aortography for verification of the urographic findings Post mortem ex

other renal function tests. An exception was case A O in whom function tests gave normal values but who showed clinical signs of renal injury with proteinuria, microhaematuria and pathological quantitative sediment. In the other six patients the creatinine clearance and/or serum creatinine and/or N P N were abnormal.

In the last case (R M) urography showed unilateral tuberculous changes which were verified at operation. The renogram showed bilateral changes but the serum creatinine and the N P N were normal.

The majority of the new cases in Table VIII were patients examined at autopsy. They have not been included in Table VII because the excretion urography could not be done owing to impairment of renal function. The causes were chronic glomerulonephritis or pyelonephritis or nephrosclerosis. All had bilateral changes on the basis of their renal diseases. All 22 cases in the series except K S had pathological values for one of the three function tests: creatinine clearance, serum creatinine and N P N (19/21, 19/22 respectively, 16/22).

In case Z N who died from a ruptured aortic aneurysm post mortem examination showed bilateral polycystic kidneys with preserved parenchyma on both sides and without any certain morphological difference between the sides. The renogram showed bilateral changes but also disparity. The bilaterally abnormal renograms corresponded to a decreased creatinine clearance of 72 ml/min and increased serum creatinine of 1.30 mg/

100 ml and N P N of 41 mg/100 ml but no explanation of the functional difference could be obtained.

Comparison between uptake ratio and other renal function tests with radioactive Hypaque

Fig. 30 a—h gives the uptake ratio for the symmetric renograms (curves without disparity) calculated as the mean of the right and left sides. This provides a possibility of obtaining measures comparable with those of other tests of total renal function. The same procedure was adopted in cases with asymmetric renograms but in Fig. 30 a—h these cases are marked (solid circles) since this procedure cannot be regarded as really correct because of the differences between the function of the two kidneys. However, most of these cases show no noteworthy deviation from the others.

Fig. 30 a—d shows that despite considerable spread of the values in several of the series there was a clear linear relation between the uptake ratio and renal function data obtained with Hypaque. The coefficient (r) of correlation between uptake ratio and clearance with intravenous infusion technique was +0.67 between uptake ratio and 2 hour urinary recovery, +0.60 between uptake ratio and retention per cent calculated from the blood curve, -0.60 and between uptake ratio and retention per cent calculated from the chest curve, -0.71. The correlation between the uptake ratio and the disappearance of ^{131}I Hypaque from the blood (plasma re-

In the three cases with normal urograms and renograms renal function was normal with the exception of N N, in whom clearance was slightly decreased (86 ml/min). The eight cases with disparity between the kidneys were identical with those cases shown in Fig. 29 where a difference was found either in the uptake or excretion ratio or in both. The ninth case was Z N, who was not examined with urography because of decreased renal function. The cases could be classified into three groups regarding the difference in uptake and/or excretion ratio. The abnormal curves were of those types (I—III) described in Fig. 26. All the cases had normal creatinine clearance, serum creatinine and N P N, except one (K M, N P N 44 mg/100 ml) and all had either morphological changes or significant differences in the size of the two kidneys.

In all ten cases with bilaterally abnormal renograms but without disparity, except case K S (horseshoe kidneys), the urograms showed poor excretion bilaterally, which corresponded well with bilateral abnormal renograms and with the other renal function tests. Of the ten nine had decreased creatinine clearance, nine increased serum creatinine and five increased N P N. Judging from the renogram, case K S had a normal uptake ratio with decreased excretion ratio, i.e. the type found in urinary obstruction but without a certain disparity between the curves. The finding corresponded well to the retarded excretion found at urography and to

other normal results of function tests.

In cases T Y, Ö N and R A with discrepancies between the urograms and renograms the renograms were normal on both sides, while urography suggested unilateral changes. In cases Ö N and R A excretion urography showed differences in the size of the kidneys with normal morphology. Supplementary aortography showed a normal vascular pattern. Post mortem examination showed no significant difference between the kidneys in these two cases except that R A had a small stone and a local pyelonephritic scar in the right lower pole but no other changes in the renal parenchyma or signs of obstruction.

Case Ö N had bilateral nephrosclerosis without any difference between the two sides. In the third case (T Y) the urogram showed double pelvis and ureters on the left side, a normal rate of excretion and a normal density on both sides. Renal function tests gave normal results and the radiographic findings were normal.

In case H S the renogram was normal and urography showed normal excretion, density and morphology but abundant small diffuse calcifications in the renal parenchyma on both sides indicating nephrocalcinosis. As expected from the normal renogram the results of renal function tests were normal.

In the seven cases where the radiographic appearance was normal on both sides while the renogram showed bilateral abnormal curves without disparity there was good agreement between the abnormal renogram and

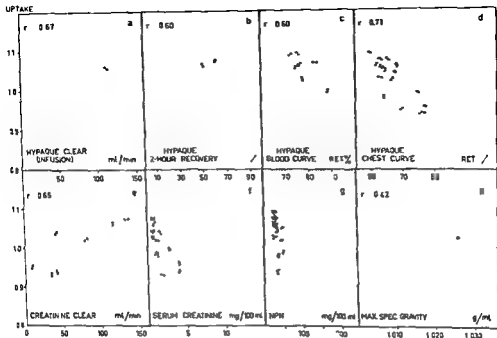


FIGURE 30 a-h Mean value of uptake ratio on both sides plotted against Hypaque clearance with infusion technique (24 cases) 2 hour urinary recovery of Hypaque (17 cases) retention per cent calculated from blood and chest curves (48 and 83 cases) creatinine clearance (48 cases) serum creatinine (43 cases) serum N P % (74 cases) and max spec. gravity (26 cases) in series A-1: solid circles Cases with symmetric renograms Open circles Cases with asymmetric renograms Hypaque and creatinine clearance are corrected to hold for 1.73 m^2 body surface

curate enough to decide whether the patient had urinary obstruction or not. Good results with this technique have been reported by Tauxe et al (1962) and Farschidpur & Schoknecht (1963).

In the investigations a rather wide collimator was used in order to allow a view of the entire kidney. This technique enables a more reliable positioning over the region of the kidney than narrow collimation; the latter method places large demands on the examiner's skill (Säterborg 1960, Johnson et al 1964, Säterborg (1960) ana-

lysed the problem of proper positioning of the detectors in renography with the aid of focusing under fluoroscopic control. With very narrow collimation (aperture of 12.5 mm) and varying centering of the detector he obtained different types of curves. Proper evaluation of the renogram requires correct centering of the detector over the kidneys, especially if such evaluation is based on measurements of amplitudes; for then small differences in the position of the detector may have a disproportionate effect on the results.

spectively chest curve), its clearance by the kidney and the urinary output showed that the parameter of the second segment of the renogram is a measure of renal function. The results also showed good agreement between these "external" and "internal" measurements with the radioactive substance

creatinine clearance, serum creatinine and serum N P N.]

Fig 31 a-f shows that the retention calculated from the external chest curve is correlated linearly with the clearance of ^{125}I -Hypaque given by intravenous infusion ($r = -0.80$), 2 hour urinary recovery of ^{125}I Hypaque ($r = -0.74$) and retention calculated from the plasma curve of ^{125}I Hypaque ($r = +0.70$). A similar relation was also found between creatinine clearance ($r = -0.75$) and a non-linear relation with serum creatinine and N P N. These correlations show that the retention parameter used is a useful supplement to renography.

E Discussion

The positions of the kidneys is determined roentgenographically in films taken with the patients sitting upright or by manual scanning after injection of ^{125}I Hypaque. Nearly all cases showed good agreement with the results obtained by control scanning after the test. Some investigators have used roentgenography and marking of the sites of the kidneys (Winter 1959; Spencer et al 1961 and others); others scanning of the back after injection of a radioactive test dose before the test (Frohlich et al 1959; Magnusson 1962; Dore et al 1963; Johnson et al 1964).

In the acute urinary obstruction series where manual scanning was used a few of the patients who were in pain moved relative to the detector. The examination could nevertheless be performed and the results were ac-

Comparison between uptake ratio and other renal function tests (creatinine clearance, serum creatinine, serum N P N and maximal specific gravity)

Fig 30 e-h gives the means of the uptake ratio in the two sides plotted against creatinine clearance, serum creatinine, serum N P N and maximal specific gravity. While the serum creatinine and serum N P N show a non-linear relation with the uptake ratio, the creatinine clearance and maximal specific gravity show a linear relation ($r = +0.65$ respectively $r = +0.42$).

These results thus point in the same direction as the above mentioned ^{125}I Hypaque data, i.e. that the uptake ratio is a useful measure in the evaluation of renal function but allows no estimation of glomerular filtration or tubular secretion separately.

Comparison between retention per cent calculated from chest curve and other renal function tests (^{125}I Hypaque clearance, 2 hour urinary recovery of ^{125}I Hypaque, retention per cent calculated from the plasma curve and

effect of the renal collimator was examined showed that the renal detectors were influenced only slightly by radioactivity from the contralateral kidney and the bladder.

One of the main questions is how the renogram should preferably be evaluated. Before attempting to answer this and related questions it must be decided which segment should be used for quantitative analysis. The results of experimental renography were discussed in Chapter VIII C. The results suggested that the first segment of the curve was less suitable while the second and third segments could be utilized best in the evaluation of the renal uptake and excretion of the substance. For analysis of the complex external renal curve a schematic diagram was given where the theoretical renal curve was said to express the net effect of the processes operating simultaneously in the kidney. The processes were summarized as inflow and outflow of the substance from the region of the kidney. In order to obtain a quantitative measure of these two processes ratios were calculated from the rising respectively falling limbs of the curve. These parameters were presumably normal or decreased and four possible combinations were obtained which represented four different types of renal curves. These four types fell into different fields in the theoretical Fig. 23 where the two parameters were plotted against each other.

When the two parameters were calculated from the renograms in the present material their distribution among the fields was largely the same

as the theoretical distribution with the exception that the values for acute and chronic urinary obstruction were not separated. The less good agreement between the theoretical requirements and the results obtained regarding this field was due to the fact that the uptake by the kidneys with chronic urinary obstruction was nearly always normal. This explains why this series did not differ from the cases in the field with acute urinary obstruction. Theoretically in a large series (including cases with reduced function) and with a more sensitive test substance than Hypaque it should be possible to demonstrate the theoretically defined relation with division of the field for urinary obstruction into two parts. It is of interest to note that neither the theoretical nor the clinical diagram contained any values in the lower right field where the curves with decreased uptake ratio and normal excretion ratio should have been. This is because the excretion ratio is lower in patients with decreased uptake ratio even if the urinary drainage is unobstructed (see page 69).

Fig. 25 contained no values in the lower left field. It should have contained curves with decreased uptake and excretion ratios. The values for excretion ratio were however situated to the right of the axis because the substance was eliminated by the contralateral kidney and gave a curve with a more rapid fall and an artificially high excretion ratio.

The results show that the theoretical model with four different types of

RETENTION % (CHEST CURVE)

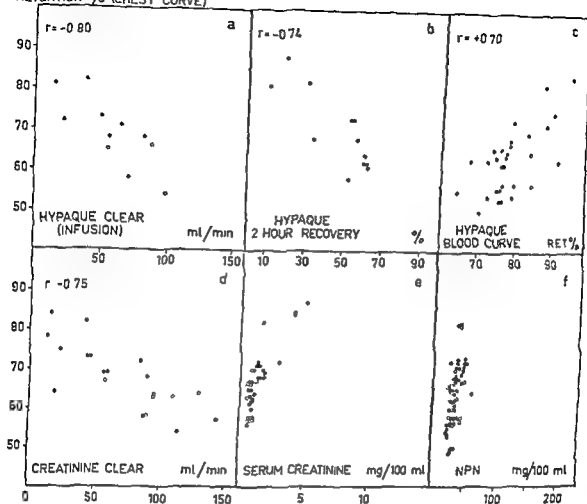


FIGURE 31 a—f Retention per cent calculated from external chest curve plotted against Hypaque clearance with infusion technique (25 cases) 2 hour urinary recovery of Hypaque (18 cases) retention per cent calculated from blood curve for Hypaque (49 cases) creatinine clearance (37 cases) serum creatinine (63 cases) and serum NPN (88 cases) in series A—F and I. Solid circles: Cases with symmetric renograms. Open circles: Cases with asymmetric renograms. Hypaque and creatinine clearances are corrected to hold for 1.73 m² body surface.

Ratios between two amplitudes are less sensitive to such changes in the position of the detector over the kidney (Spencer et al 1961 Stewart & Haynie 1962, Wax & McDonald 1962 Krosgård & Frus 1964). As discussed in Chapter VIII C the lack of a standard technique of renography is to no small extent responsible for the

very divergent results reported in the literature. Some authors question whether the renogram allows quantitative analysis of renal function (Spencer et al 1961 Pircher et al 1963).

Our technique has however given fairly reproducible results and the model experiments where the shielding

Figs 30 a and c that cases were seen with about 50 per cent impairment of function (^{131}I Hypaque and creatinine clearance) with an uptake ratio that lay within the normal range. This means that ^{131}I Hypaque does not satisfactorily differentiate between diseased and normal kidneys. Other substances such as ^{131}I Hippuran with its high clearance and high concentration in the kidney which is reflected by the slope of the second segment of the renogram should provide a much better possibility for obtaining a measure with a narrower range. There should be a much smaller risk of overlap between the pathological and normal curves and thereby the value of these external measurements is increased.

A number of investigators have compared the renogram and different renal function tests. Serratto et al (1959) reported that blood urea between 16—20 mg/100 ml is not uncommon in patients with a normal ^{131}I Diodrast renogram. When the blood urea exceeded 20 mg/100 ml the incidence of bilaterally abnormal renograms was high (27/32). Normal ^{131}I Diodrast renograms were not uncommon in patients in whom the urea clearance was markedly reduced. Substantially decreased urea clearance was however always associated with bilaterally abnormal renograms. Oeff et al (1963) found that the ^{131}I Hippuran renogram could be normal even when the PAH and inulin clearance were decreased. They recommended that if one wanted to examine slight impairment of renal function clearance tests should be used because the renogram

does not reveal mild impairment. Similar experience has been made with radioactive Diodrast and Hippuran renogram by Roth et al (1960), Stratton & Garcia (1960), Cobb (1961), Minami et al (1961), zum Winkel et al (1961 c), Scholz et al (1964) and zum Winkel (1964).

A supplementary parameter mainly to the uptake ratio is the retention calculated from the external chest curve. The linear relation between retention and renal function data for ^{131}I Hypaque and creatinine clearance shows that retention could be used as a measure of renal function. Retention together with the external renal measurements makes it possible to estimate total and individual renal function. Such types of parameters have been used by Winkler (1961) and zum Winkel (1964).

One of the most important purposes of renography is to compare the two sides and show any disparity between them. In Figs 27 and 28 it was easy to compare not only the individual but also the pairs of values in the individual patients. This form of diagrammatic illustration allows direct evaluation of the distance between the values on the sides compared and at the same time their localisation within the fields. The procedure implies a considerable simplification and facilitates comparisons between the different series of diseases. This evaluation with only two parameters calculated from the second and third segments of the curves can be used also for renograms made with other substances such as ^{131}I Hippuran. One can extend the

renal curves distributed among different fields agrees with the localisation of the renograms from the clinical series. This agreement means that parameters calculated from the theoretical renal curve can be applied to the more complex renogram and thus be suitable for clinical use.

The fields obtained from the clinical series then offer a possibility to determine the individual ratios for each case in the series with and without renal disease. It is clear from the discussion in Chapter VIII C where a number of evaluation techniques were described, that evaluation of the renograms by visual inspection alone is not satisfactory. This technique implies about all a risk of subjective interpretation by the examiner. It should be underlined, however, that one must consider the general shape of the curve and any changes possibly due to less satisfactory conditions during the examination (changes in patient's position because of pain, or nervousness etc.).

Parameters calculated as relative values from the renogram proved the most suitable provided that their evaluation was not too complicated and laborious for routine clinical use. It is also of importance that the parameters can be calculated from all types of curves which is difficult in *inter alia* slope analysis with tangents or determination of t_{\max} (Brown et al 1963, Krosgaard & Ivers 1964). As discussed in Chapter VIII C t_{\max} has, however, often been used as a parameter. It is probable that this parameter, though dependent on both

uptake and excretion function, may be valuable in comparison between the two kidneys of a given patient (Scholz et al 1964). t_{\max} was found less suitable for the ^{131}I Hippuric renogram, but may be more informative in renograms made with substances with high clearance rate e.g. ^{131}I Hippuran (zum Winkel et al 1961 c, Stewart & Haynie 1962, Wax & McDonald 1962).

In order to find out what the renogram measures, the linear relation between the uptake ratio and renal function data obtained with ^{131}I Hippuric and creatinine clearance respectively, maximal specific gravity, showed that the choice of a ratio calculated from the second segment was justified as a measure of renal function. This ratio, however, gives no numerical value for glomerular filtration or tubular secretion separately. The same good relation between the parameters applied to the second segment and renal function data have been reported for ^{131}I Hippuran by Becker et al (1963), Wax & McDonald (1964) and zum Winkel (1964) while Taplin et al (1963) found a good correlation between renal blood flow (clearance of Radio Hippuran) and the slope of the second segment. Block et al (1964) found a less good correlation between uptake $\Gamma 1/2$ of the second segment of the ^{131}I Hippuran renogram and PAH clearance while ^{131}I Diodrast had formerly been found to show a good correlation between this parameter and the 30 minute excretion of ^{131}I Diodrast (Block et al 1960 a).

As to the sensitivity of the ^{131}I Hippuric renography it is clear from

Figs 30a and c that cases were seen with about 50 per cent impairment of function (^{125}I Hypaque and creatinine clearance) with an uptake ratio that lay within the normal range. This means that ^{125}I Hypaque does not satisfactorily differentiate between diseased and normal kidneys. Other substances such as ^{125}I Hippuran with its high clearance and high concentration in the kidney which is reflected by the slope of the second segment of the renogram should provide a much better possibility for obtaining a measure with a narrower range. There should be a much smaller risk of overlap between the pathological and normal curves and thereby the value of these external measurements is increased.

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A supplementary parameter mainly to the uptake ratio is the retention calculated from the external chest curve. The linear relation between retention and renal function data for ^{125}I Hypaque and creatinine clearance shows that retention could be used as a measure of renal function. Retention together with the external renal measurements makes it possible to estimate total and individual renal function. Such types of parameters have been used by Winter (1961) and zum Winkel (1964).

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procedure by giving the results as percentages of the normal values of the parameters and obtain a numerical relation between the kidneys

In the comparison between urograms and renograms the series with parenchymal renal disease, including the hypertension series, agreement was found in 21 of 33 cases. On comparison of the renographic findings with the results of urography, hiparotomy and post mortem examination the corresponding values were 33 of 44

The lack of agreement can be explained by the fact that urography and renography are not strictly comparable from a functional point of view. The extensive use of excretion urography in clinical investigations of renal diseases, however justifies such a comparison. If the renogram is abnormal in a patient without urographically demonstrable changes, it does not necessarily mean that the renogram is wrong, but simply that there is no close correlation between the two methods

Among cases with a discrepancy between the tests there were seven where the urograms were normal, but the renograms bilaterally abnormal without any difference between the sides. In six of the cases, however the total function was decreased which corresponded to the renogram. The urograms was thus less sensitive to impairment of renal function, which is in agreement with the results reported by Edling et al (1956) and Squire & Schlegel (1959), who compared the urographic findings with selective clearance determinations *e.g.* regard

ing PAH and inulin. Similar results with normal urograms and bilateral abnormal renograms where the total function corresponded to the renograms have been reported by Serratto et al (1959), Strifflon & Garcia (1960), Winter (1960) and zum Winkel (1964). In the other cases with a discrepancy between the tests, it could be explained by insignificant morphological changes in the urogram not having produced any impairment of renal function

In the series not examined with urography because of impaired renal function but examined post mortem good agreement was invariably found between the bilateral changes demonstrated by the renogram and the results of autopsy. The renograms also showed agreement with results from renal function tests

In the eight cases where the renogram showed disparity between the kidneys the corresponding urogram revealed either morphological changes with poor density in the pelvis or significant differences in the size of the kidneys. In this series it was remarkable that total renal function determined by creatinine clearance, serum creatinine or N P N provided no guidance. These values were normal in all the patients and thus give no information about the differences between the two kidneys in each patient. Determination of total function by clearance tests is thus of limited value in deciding whether a patient has unilateral impairment of renal function and in such cases the renogram may prove a helpful guide to the clinician

in the further investigation of the case (roentgen examinations and selective clearance tests)

While comparison between urography and renography in the above mentioned series was complicated by the fact that urography has certain drawbacks as a function test, the relationship was better in the evaluation of different forms of urinary obstruction. Since its introduction in the 1930s excretion urography has proved the most reliable method for demonstrating urinary obstruction especially in acute situations but also in chronic urinary obstruction (Borminghaus 1932 Hellmer 1935 Wulff 1936 and others). Good agreement was found between the clinical and roentgenological findings both in the acute stage and in the later follow up. As far as renography is concerned the most important question is whether the method and especially its parameters (excretion ratios $1\frac{1}{2}/30$ and $5/1\frac{1}{2}$ see page 67) are sensitive enough to demonstrate urinary obstruction in the acute stage as well as in the chronic stage of urinary obstruction of varying origin. The choice of excretion ratio $1\frac{1}{2}/30$ was therefore made in order to obtain as true a renographic measure as possible of the elimination of the test substance from the area of the kidney.

It is clear from Fig. 271 that the values found for cases with urinary obstruction fell within the upper left field. The values for the contralateral healthy side and cases where obstruction could be excluded were mostly within the normal field. The difference

in excretion ratio was sufficient to eliminate the roentgen verified cases of urinary obstruction.

While the difference in excretion ratio is most useful in deciding whether a patient has urinary obstruction or not the difference in uptake ratio is of importance because it also reveals any difference in function between the two kidneys. The patients in the acute obstruction series with difference in uptake ratio belonged to the group with pain during the test and therefore unable to sit still. This may explain the difference in uptake since the patients had no history of renal disease. In the present investigation urinary obstruction was the only disease that caused a typical change in the appearance of the renogram with a decreased excretion ratio provided that uptake function was normal. If urinary obstruction can be excluded, the renogram reflects only the uptake function. These results are in agreement with those reported by Burbank et al (1963), Farmelant et al (1964), Scholz et al (1964) and zum Winkel (1964).

It should however be underlined that this appearance of the renogram is also seen in other states such as a low rate of urine flow of varying cause (Wedeen et al 1963). This type of curve has been produced experimentally and seen clinically in severe dehydration and in a faint (Winter 1956 1963 O'Connor et al 1961 Way & McDonald 1962). In the present material the patients had moderate hydration and were not intentionally dehydrated. Exceptions to this rule were

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cases with suspect urinary obstruction where the patients did not drink before the examination. This series thus included patients with pain, nausea and vomiting, without clinical signs of any severe dehydration or blood pressure fall resembling shock. The good agreement between the renograms and urograms did not provide any evidence for "false" positive obstruction curves due to extreme dehydration.

Renography showed that it was in chronic pyelonephritis, hypertension, other renal parenchymal disease and in polycystic disease that disparity was found between the two kidneys in the series with renal parenchymal disease. These results are in agreement with those reported by other investigators (Winter 1956, 1957, Winter et al 1959, Block et al 1960, zum Winkel et al 1961c, Dore et al 1962, Stewart & Hayne 1962, Burbank et al 1963, Schutterle & zum Winkel 1963, Firmefant et al 1964, Scholz et al 1964, zum Winkel 1964).

One of the most important points discussed in the literature is whether the renogram is of any value as a screening test for demonstrating unilateral renal injury in hypertension, where renal changes are due to stenosis of the renal arteries or parenchymal damage, such as unilateral chronic pyelonephritis. Since the first preliminary publications by Winter (1956, 1957) the literature in this field has become voluminous. Most of the publications are however confined to case reports, large series still being few.

Favourable results have been presented by several groups of workers who used radioactive Diodrast, Hypaque or Hippuran renograms alone or combined with urography with rapid film technique in the screening of the patients with unilateral vascular disease from other cases with hypertension (Winter et al 1959, Block et al 1960a, Maxwell et al 1960, Burrows et al 1961, Tausk 1961, Hunt et al 1962b, Maxwell 1962, Whitley et al 1962, Burbank et al 1963, Dore et al 1963, Maxwell et al 1964, Wall & Whalen 1965).

According to Stewart & Hayne (1962) the ^{131}I Hippuran renogram is ineffective as a screening test since it gave a high percentage of false positive (25%) and negative (27%) results. Similar experience has been reported by Scott & Quesada (1962) and Truss & Krogsrud (1964). One possibility of reducing the frequency of false positive and negative results would be to use an evaluation technique according to the principles described above. In the material presented cases with different renal diseases have been included to exemplify the renograms in these diseases. The material is too small to allow any conclusions about the frequency of certain pathological patterns in the different groups of diseases.

In recent years the use of the renogram showing different forms of urinary obstruction has received increasing attention. In series with acute suspect urinary obstruction the renogram was able to distinguish patients with different abdominal conditions

from cases with urinary obstruction due to stone. The renogram has thus proved to be a valuable supplement to other examination methods. Parts of the present material have been described in preliminary studies and the results have shown that the renogram is a good supplement to excretion urography (Denneberg 1959/60). The renograms proved especially valuable in those cases where the urography could not give any definite information in cases of suspected urinary obstruction without verified stone or in so called roentgenologically silent kidney. Other investigators have shown that patients with ureteric stone can be followed with advantage by serial renography (Boyd et al 1959 Serrallo et al 1959 Lawrence et al 1963 zum Winkel 1964).

Of interest was case M J in the series with acute suspected urinary obstruction. Since the patient had only one kidney urography was not done because of the risk of complications. The renograms showed no signs of obstruction. Follow up after the acute stage verified that it was not a question of urinary stone but of cystopyelitis. A related problem is that urographic investigation is contraindicated by an increased $\Delta P \Delta$. Excretion urography with the dose necessary for roentgen examination makes such an examination hazardous besides which the quality of the films is less satisfactory. Recording of the renogram however is not attended by any risk and an increased $\Delta P \Delta$ does not contraindicate the examination. It has been shown experimentally and clinically

that renography can distinguish between acute anuria and oliguria due to acute bilateral obstruction, acute tubular necrosis and acute dehydration during the first 48 hours (O'Connor et al 1961 Wax & McDonald 1962 Lawrence et al 1963 Ross et al 1963 Trinkle & Kiser 1964).

The present series of acute urinary obstruction as well as in the hydro-nephrosis series included examples of so called roentgenologically silent kidneys (non visualized kidneys). This term is not tantamount to non functioning kidney but in a roentgenological classification of kidneys without uptake and excretion of contrast medium at urography. Clearance examinations can be difficult to perform because little or no urine is excreted. In such cases the renogram can give information on the possible uptake capacity of the kidney. While this roentgenological picture is not uncommon in acute urinary obstruction (Olsson & Jönsson 1962) Spjut & Nicolai (1961) found that very few non visualized kidneys were completely normal. Obstruction of the urinary tract and acute or chronic pyelonephritis were the commonest causes.

Irrespective of the cause of the silent kidney such a finding leads to an extensive urologic investigation with cystoscopy, ureteric catheterisation, retrograde pyelography and renal angiography. If the renogram in such cases shows signs of obstruction it is a sign that the kidney can recover its function after elimination of the obstruction which has been shown experimentally in animals by Denneberg

et al (1961 b) and zum Winkel (1964)

In cases with non visualized kidney in the acute urinary obstruction series the renogram showed continuously ascending curves and pathologically decreased excretion ratios as a sign of the urinary obstruction. On the other hand the two cases with silent kidneys in the hydronephrosis series (cases T J and E H), showed no such uptake segment with a continuously rising curve as a sign of urinary obstruction, but only a falling curve of the type seen in Fig. 26, i.e. a kidney without function, which was verified at angiography and autopsy. In the cases in the hydronephrosis series (cases K O D D, V P, J W and B P) the renogram proved valuable as a supplement to the morphological roentgen examination in deciding whether operation should be performed or not. It should be underlined that the renogram offered a possibility of evaluating both the diseased and the healthy side. The function of the healthy kidney is of significance in contemplated nephrectomy of the diseased kidney (Hauge et al 1962, Lawrence et al 1963, Gasser & Hawliczek 1964). The value of the renogram in postoperative gynaecological complications of the urinary tract (silent ureteral injury and abscess or cancer infiltration) can be exemplified by cases S A and E E in the hydronephrosis series. The renogram supplemented urography by

showing preserved capacity of uptake and evidence of the functioning parenchyma. Good experience with ^{131}I Hippuran renography in gynaecological diseases and in complications of the urinary tract have been reported by various authors (Gerbie et al 1961, 1962, zum Winkel et al 1961 a, b, Quinn et al 1962, Dische et al 1963, West & Nordyke 1963, Roddick et al 1964, zum Winkel 1964).

It is apparent from the data given above that the evaluation procedure with two parameters calculated from the second and third segments of the renogram provides a simple method for estimating the function of the individual kidney in clinical practice. The clinical examples illustrate the value of renography in the investigation of various renal diseases and especially for demonstrating functional disparity between the kidneys in hypertension, chronic pyelonephritis, other renal parenchymal diseases, polycystic disease and in urinary obstruction. Since renography allows examination of the function of each kidney separately, it is a valuable supplement to conventional clearance tests which measure only the total renal function and which are therefore of less value in the investigation of unilateral renal disease. Renography is also a useful supplement in cases where excretion urography cannot give sufficient information.

GENERAL SUMMARY

Hypaque (sodium diatrizoate) is widely used as a contrast medium for excretion urography. Labelled with ^{131}I it is also used for external renal measurements. The purpose of the present investigation was to elucidate the distribution and excretion of radioactive Hypaque and its use in radioisotope renography. The material consisted of 183 hospital patients with and without renal disease and 10 healthy students.

After a single injection in normals the plasma curve fell rapidly and after two hours more than 90 per cent of the radioactivity had left the blood. This rapid elimination from the blood corresponded to the rapid excretion of the substance in the urine. The extra renal excretion of the substance is normally negligible as seen *inter alia* from the low urinary 24 hour excretion. The 2 hour distribution volume of ^{131}I Hypaque was on the average 20 per cent of body weight.

^{131}I Hypaque clearance was studied after simultaneous administration of inulin, PAH and radioactive Hypaque with addition of varying amounts of non radioactive Hypaque with and without blocking of the tubules by Probenecid. The mean ^{131}I Hypaque clearance was 115 ml/min with an ob-

served range of 80—153 ml/min in the controls. The clearance values were not changed when the initial plasma level of Hypaque was varied between 0.002—6 mg/100 ml plasma.

It is known that Hypaque binding to serum proteins is negligible in man which means that the substance is completely filtrable via the glomerulus. The question whether also tubular secretion occurs was studied by comparing the clearances of ^{131}I Hypaque and inulin. The mean quotient between the clearances of the two substances was greater than 1.0 suggesting tubular secretion of ^{131}I Hypaque. Observations also argued for an almost linear relation between the Hypaque/inulin quotient and inulin clearance which at high inulin clearance gave a quotient of 1.0 or less. After administration of Probenecid the Hypaque/inulin quotient decreased indicating blocking of the secretion of ^{131}I Hypaque. On the basis of these results it was concluded that ^{131}I Hypaque is eliminated largely by glomerular filtration but also to some extent by tubular secretion. Certain observations also argue for the occurrence of tubular reabsorption. The two last mentioned processes compensate one another.

more or less completely. This means that even though ^{131}I -Hypaque is not a true measure of glomerular filtration, it should be clinically useful as a substitute for inulin clearance.

To find out the most suitable technique for measurement of single injection clearance three types of such clearances (total, 2- and 24 hour renal clearances) were compared with intravenous infusion clearance. This comparison showed a good correlation with the 2- and 24 hour clearances, while the values obtained for total clearance were systematically too high because the plasma curve for ^{131}I Hypaque had not reached its final slope at 2 hours. The good agreement suggests that the 2- and 24 hour clearances could replace the more complicated clearance with the infusion technique, besides which it can be done simultaneously with renography.

A new method of evaluation of the external renal curves (renograms) is presented. The processes in the renal handling of the test substance were summarized as inflow and outflow of the substance from the region of the kidney. In order to obtain a quantitative measure of these two processes ratios were calculated from the rising (uptake ratio) and falling (excretion ratio) limbs of the curve. These parameters may be either normal or decreased and four possible combinations are thus obtained which represent four different types of functional disturbances. The four types fall into different fields when the two parameters are plotted against each other in a diagram. The appearance

of the renogram is also affected by extra renal factors (the radioactivity in the blood, extra vascular space and bladder). Even when the two ratios were calculated for patients with a known type of renal functional disturbance, the position of the parameters in the diagram were in agreement with the above theoretical analysis.

That the uptake ratio is a good measure of parenchymal function of the kidney is apparent from the good correlation found between this parameter and renal function data obtained with radioactive Hypaque (clearance with infusion technique, 24 hour urinary recovery, disappearance from the blood) and other tests on renal function (creatinine clearance, serum creatinine, N P N and maximal specific gravity).

That the excretion ratio reflects changes in the elimination from the kidney was shown by the fact that this ratio could distinguish cases of urinary obstruction (acute or chronic) from unobstructed cases. The parameters selected and the diagrammatic presentation of their values therefore appear to be useful in the evaluation of the renogram in clinical practice.

One of the most important purposes of renography is to compare the two sides and show any disparity between them. The proposed diagram allows easy evaluation and comparison not only of the individual but also of the pairs of values in a given patient. This procedure also facilitates comparison between the different types of renal diseases. This evaluation with only

two parameters calculated from the second and third segments of the curves can be used also for renograms made with other labelled substances

Renographic disparity between the two kidneys was demonstrated by differences between the uptake or excretion ratio on either side. Such disparity was found in hypertension, chronic pyelonephritis, other renal parenchymal diseases and in polycystic disease.

A comparison between the renographic findings and findings at urography, laparotomy and autopsy showed agreement in two thirds of the cases with parenchymal renal disease including hypertension. The lack of agreement in the remaining third could partly be explained by the fact that urography and renography are not strictly comparable from a functional point of view, as was shown in cases with decreased clearance values having bilaterally pathological renograms but normal urograms.

Cases with renographic disparity had urographic signs of morphologic changes with poor density in the pelvis or significant differences in the size of the kidneys. Total renal function determined by clearance tests was normal and yielded no information about the differences between the two kidneys in a given patient. Determination of total renal function is thus of limited value in deciding whether a patient has unilateral impairment of renal function and in such cases the renogram is useful in the further investigation.

In the remaining cases with discrepancy between the tests this could be

explained by small morphological changes in the urogram not having produced any impairment of renal function. In the series not examined with urography because of impaired renal function but examined post mortem good agreement was invariably found between the bilateral changes demonstrated by the renograms and the results of autopsy and renal function tests.

In the series with suspected acute urinary obstruction the renogram was able to distinguish patients with various abdominal conditions from patients with urinary obstruction due to stone. Renography thus proved to be a valuable supplement to other renal examinations. Renography also proved valuable in those cases where excretion urography could not give any definite information in cases of suspected urinary obstruction without verified stone or in roentgenologically silent kidneys. It also proved useful to estimate renal function on both sides before contemplated nephrectomy for hydronephrosis and at follow up of renal function after gynaecological operations with complications from the urinary tract.

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DETERMINATION OF CLEARANCE AND DISTRIBUTION VOLUME WITH THE SINGLE INJECTION TECHNIQUE

by H NOSSLIN

(Department of Chemical Chemistry General Hospital, Malmö Sweden)

Theoretical analysis of the kinetics of a substance deposited in an open biological system by single injection has been the subject of numerous papers since Teorell's (1937 a and b) publication of the fundamental principles. Reviews of compartment analysis have been given by Dost (1953) Sheppard (1962) and Riggs (1963). The theoretical treatment of the clearance problems dealt with here is based on these conventional principles but is at the same time focused especially on those problems encountered in the analysis of data obtained in experiments with test substances eliminated entirely or partly by the kidneys.

Symbols

- t = time (min)
 P = total amount in plasma (fraction of dose)
 V = plasma volume (ml)
 D = distribution volume (ml)
 Q = total amount excreted from plasma (fraction of dose)
 U = total amount excreted by renal clearance (fraction of dose)
 C = total clearance from plasma (ml/min)

- C_R = renal clearance from plasma (ml/min)
 F = urinary flow (ml/min)
 S = renal tract dead space (ml)
 k_P = fractional rate of total plasma clearance = C/V (min^{-1})
 k_R = fractional rate of renal plasma clearance = C_R/V (min^{-1})
 A = total area under the P curve (min)
 A_t = area under the P curve to time t (min)
 c = coefficient of exponential term describing the P curve (fraction of dose)
 b = exponential constant in the expression for the P curve (min^{-1})

Assuming that the injected substance mixes immediately with the plasma the initial theoretical concentration of the substance will be the value obtained on division of the dose by V . This value is taken as 1 and all later values are expressed with reference to this value and thereby show what proportion of the dose is circulating in the plasma at the time of sampling.

It is also assumed that the plasma curve can be described by an arbitrary number of exponential functions

$$P = c_1 e^{-b_1 t} + c_2 e^{-b_2 t} + \dots = \sum c e^{-bt}$$

The total area under the P curve is obtained by integration of this expression between $t = 0$ and $t = \infty$, which gives

$$A = \frac{c_1}{b_1} + \frac{c_2}{b_2} + \dots = \sum \frac{c}{b}$$

The area under the P curve for a definite time (A_t) can be calculated either as the difference between A and the remaining area after time t (calculated in analogous manner), or by direct measurement by some conventional method (planimetry, weighing, counting of squares, numerical calculation with integration formulae etc.)

I Total plasma clearance

Since the total excretion must equal total clearance we get

$$dQ/dt = K_P P$$

Integration gives

$$Q = K_P \int P dt$$

After an infinite period the entire dose is excreted which gives

$$1 = K_P \int_0^{\infty} P dt = K_P A$$

Since K_P according to definition is $= C/V$ we finally get

$$C = \frac{V}{A} \quad (1)$$

The total clearance is thus calculated as the quotient between the plasma

volume and the total area under the plasma curve. This formula holds for all substances added to the plasma (whether as a single injection or by infusion) and is independent of the co-occurring distribution between the plasma and the extravascular space. The only requirement is that clearance is constant during the observation period. When P is expressed as a fraction of the dose, the area A has the dimension time. It can be shown that this time is the mean time the substance remains in the plasma. It corresponds to the turnover time in kinetic analysis of metabolic processes.

II Renal clearance (uncorrected for dead space)

This clearance is calculated analogously to that for total clearance

$$dU/dt = K_R P$$

Integration gives

$$U = K_R \int P dt$$

At time t

$$U_t = K_R \int_0^t P dt = K_R A_t$$

Since $K_R \approx C_R/V$ we get

$$C_R = \frac{U_t V}{A_t} \quad (2)$$

However in practice this formula gives systematic errors since U_t refers to all substance that has been excreted by the kidney i.e. the sum of the amount in the renal tract plus the amount in the bladder. Since only the amount that has reached the bladder

can be measured the value observed is too small and C_R correspondingly too low

III Correction for renal tract dead space delay

This dead space has two effects, both causing a delay in the excretion measured namely a transport delay because of the time the substance needs to run through the urinary tract and secondly a mixing delay because during its passage newformed urine is mixed in the dead space with previously secreted urine. An adequate correction should cover both types of delay (Bojesen 1954). With the aid of the known urine flow a simple approximate calculation of this correction can be done as follows

- Transport delay As pointed out by McSwiney & DeWardener (1950) this may be conceived as the quotient between the dead space volume and the urine flow i.e. delay time is S/F
- Mixing delay This can also be regarded as a function of dead space volume and urine flow. Bojesen (1954) discussed this problem in detail and showed that with a linearly falling plasma curve the mixing delay time is S/F for an exponentially falling curve the corresponding expression is $S/F (1 + 1/2)$. When the curve falls slowly the difference between the two expressions is negligible.

The total delay correction may thus be taken as $2 \times S/F$. Generally only F is known so that S must be estimated.

McSwiney and DeWardener (1950) determined the minimum transport time at different urine flows and showed that the calculated dead space varied regularly with the magnitude of the flow which is also apparent from their Fig 1. Bojesen (1954), who used dogs showed that, except when the urine flow is very high or very low the dead space volume increases linearly with the flow.

The following regression formula was calculated from data given by McSwiney and DeWardener (3 cases with lowest rate of urine flow excluded)

$$S = 3.28 + 1.82 F$$

This gives the following expression for total delay correction

$$T = 2 S/F = 3.6 + 6.0/F \quad (3)$$

This formula holds when the urine is collected under the conditions used by McSwiney & DeWardener (1950), i.e. continuous collection via catheter. The dead space of the catheter (2 ml) is thus included in the calculated dead space volume. The following values for total correction for different urine flows are obtained

Flow (ml/min)	Correction (min)	Flow (ml/min)	Correction (min)
1.0	10.2	5.0	4.9
1.5	8.0	6.0	4.7
2.0	6.9	7.0	4.5
2.5	6.2	8.0	4.4
3.0	5.8	9.0	4.3
4.0	5.3	10.0	4.3

When the bladder urine is collected intermittently (spontaneous voiding or by catheter with rinsing) the volume

It is also assumed that the plasma curve can be described by an arbitrary number of exponential functions

$$P = c_1 e^{-b_1 t} + c_2 e^{-b_2 t} + \dots = \sum c e^{-bt}$$

The total area under the P curve is obtained by integration of this expression between $t = 0$ and $t = \infty$, which gives

$$A = \frac{c_1}{b_1} + \frac{c_2}{b_2} + \dots = \sum \frac{c}{b}$$

The area under the P curve for a definite time (A_t) can be calculated either as the difference between A and the remaining area after time t (calculated in analogous manner), or by direct measurement by some conventional method (planimetry, weighing, counting of squares, numerical calculation with integration formulae etc.)

1 Total plasma clearance

Since the total excretion must equal total clearance we get

$$dQ/dt = K_P P$$

Integration gives

$$Q = K_P \int P dt$$

After an infinite period the entire dose is excreted which gives

$$1 = K_P \int_0^{\infty} P dt = K_P A$$

Since K_P according to definition is $= C/V$ we finally get

$$C = \frac{V}{A} \quad (1)$$

The total clearance is thus calculated as the quotient between the plasma

volume and the total area under the plasma curve. This formula holds for all substances added to the plasma (whether as a single injection or by infusion) and is independent of the co-occurring distribution between the plasma and the extravascular space. The only requirement is that clearance is constant during the observation period. When P is expressed as a fraction of the dose, the area A has the dimension time. It can be shown that this time is the mean time the substance remains in the plasma. It corresponds to the turnover time in kinetic analysis of metabolic processes.

II Renal clearance (uncorrected for dead space)

This clearance is calculated analogously to that for total clearance

$$dU/dt = k_R P$$

Integration gives

$$U = k_R \int P dt$$

At time t

$$U_t = k_R \int_0^t P dt = k_R U_t$$

Since $k_R = C_R/V$ we get

$$C_R = \frac{U_t V}{A_t} \quad (2)$$

However in practice this formula gives systematic errors since U_t refers to all substance that has been excreted by the kidney i.e. the sum of the amount in the renal tract plus the amount in the bladder. Since only the amount that has reached the bladder

arterial value. The higher the clearance rate the more pronounced this difference (Brun et al 1949). Since renal clearance occurs from arterial blood, arterial or capillary blood should be used for determination of the plasma level. If venous blood is used, the value obtained for the area A might be too small, which according to the formula (1)–(3) results in too high a value for clearance. However, the longer the venous curve is followed, the smaller this error, since negative and positive differences tend to compensate one another. There is theoretical evidence that this compensation is complete if the venous curve is calculated to infinity. It should thus be possible to obtain a correct value for the total clearance from measurement of venous blood according to the formula (1) and a correct value for renal clearance according to formula (3) if the urine is collected for a long time, e.g. 1 day.

In tests with some substances the plasma curve may require a long time to reach its true final slope. If sampling is ceased before it has reached its final slope and the then final part of the curve is extrapolated to infinity, the area under the curve will be too small and the total clearance value too large. At the same time the calculated total distribution volume will

be too small. The shorter the time the plasma curve is followed, the greater the error. It is thus better to sample at relatively long intervals for a long time instead of at short intervals for a short time. Calculation of renal clearance according to formula (3) avoids this source of error because with this formula one can use a directly measured limited part of the total area under the plasma curve. This possible source of error of the total clearance should be borne in mind in the calculation of extrarenal clearance as the difference between total and renal clearance.

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of the catheter is not included with the dead space and the expression for dead space should be reduced by 2 ml

$$S = 1.28 + 1.82 F$$

$$T = 3.6 + 2.6/F \quad (4)$$

The total correction will then be

Flow (ml/min)	Correction (min)	Flow (ml/min)	Correction (min)
1.0	6.2	5.0	4.1
1.5	5.3	6.0	4.0
2.0	4.9	7.0	4.0
2.5	4.6	8.0	3.9
3.0	4.5	9.0	3.9
4.0	4.3	10.0	3.9

The correction, as expected, is greatest at low flow and at high flow it asymptotically approaches the limit of 3.6 min. The larger the flow rate the smaller effect of the dead space of the catheter. At a urinary secretion rate of about 5–10 ml/min the correction is about 4 min which is somewhat more than the 2–3 min which have so far often been used and which correspond to "first appearance time" + t_e transport delay.

The formulae for delay correction show the importance of a high urine flow in clearance studies. Moreover, in tests using the single injection technique the relative importance of correction decreases with increasing duration of the collection period used. High urine flow and a long collection period are therefore desirable.

IV Renal clearance (with correction for dead space)

The time correction calculated in the previous section should by rights be

applied in the form of a prolongation of the time for the collection of urine. If the plasma disappearance curve and the curve for the excretion rate are parallel, correction may equally well be done by a corresponding subtraction of time in reading the plasma value. This is in practice simpler, since the plasma curve is followed continually. If the urine is collected from the beginning of the test to the time t , the area under the plasma curve should thus be measured to time $t + T$. The formula for renal clearance is then

$$C_R = \frac{U_t V}{A_{t+T}} \quad (5)$$

V Distribution volume

If the plasma curve is followed until it reaches its final slope and is resolved into exponential terms, the total distribution volume can be calculated according to the following formula

$$D = V \frac{\sum (b/c^2)}{(\sum b/c)^2} \quad (6)$$

This formula holds for all substances that are not eliminated directly from the extravascular space. The derivation of the formula is given in Andersen (1964 Appendix G).

VI Comments

In all tests with the single injection technique there is an arteriovenous difference in the plasma values. In the beginning the venous value is lower than the arterial value; it becomes momentarily identical with and then remains constantly higher than, the

APPENDIX
(Tables IX-XX)

Table IV. Exponential constants (c) and intercepts (b) found by slope analysis in 31 cases used in study of clearance of H₂paque with single injection technique (Chapter VII)

Case	Intercepts				Exponential constants			
	c ₁	c ₂	c ₃	c ₄	b ₁	b ₂	b ₃	b ₄
LN	0.162	0.132	0.211	0.495	0.00737	0.0667	0.230	1.330
III	143	157	309	391	772	426	344	1.262
SP	159	142	255	414	686	529	356	1.228
KA	172	196	225	407	937	672	297	0.849
DA	144	157	353	346	711	545	356	0.796
WJ	167	227	606		866	698	495	
LL	135	133	255	477	745	458	245	0.939
HB	171	176	206	447	704	694	329	1.146
AJ	143	165	265	427	987	635	279	1.108
EH	206	181	613		1005	658	313	
SN	161	134	314	391	845	579	191	0.831
SL	204	299	497		963	680	431	
HVI	103	163	294	380	783	509	307	1.064
VI	157	157	323	363	892	590	237	0.887
VO	225	191	274	310	726	529	301	1.425
RA	127	096	038	679	630	529	181	1.044
BE	159	112	193	536	927	536	177	0.939
BA	167	159	230	444	737	606	354	2.494
GA	174	076	279	473	779	512	186	1.064
IIJ	225	284	491		555	931	939	
IG	176	134	216	474	663	480	242	1.471
IS	183	274	353	190	758	980	356	2.763
WA	174	122	245	459	529	485	237	0.798
LB	167	142	274	417	571	447	270	1.413
AA	173	152	057	618	419	456	135	0.458
IG	202	186	167	445	508	837	419	1.257
KH	206	046	142	606	360	409	134	0.783
VO	197	304	270	229	258	954	307	0.939
LA	230	113	216	441	219	614	230	1.124
TB	288	157	304	251	250	470	237	0.854
BI	257	056	221	436	184	731	341	0.979

Table V. Urine recovery, clearance of Hypaque with single injection and intravenous infusion technique and distribution volume in 31 cases (Chapter VII)

Case	Urine flow ml/min	Urine recovery		Renal clear ¹		Total ¹ clear	Infusion ¹ clear	Distr volume
		2 h %	24 h %	2 h ml/min	24 h ml/min	ml/min	ml/min	ml/min
EN	2.1	51	95	120	141	149	129	17300
IH	1.9	62	96	120	125	130	124	13500
SP	3.6	54	95	112	122	128	124	16100
KA						161		14300
DA						154		18300
WJ						146		14200
LL						108		12000
HB						86		11000
AJ			98		162	165		13500
EK			96		132	138		11400
SN			91		115	126		12400
SA			95		96	101		8600
HM			95		95	100		10700
ML	0.4	62	93	147	147	158	131	14600
AO	3.0	60	102	99	102	102	116	12100
RA	2.8	48	95	99	114	120	108	16800
BE	1.8	60	93	110	119	128	107	11600
BA	0.4	50	91	105	108	119	107	14300
GA						124	87	14000
HJ	1.9	43	86	81	82	96	86	15900
HG	2.7	56	97	77	79	81	78	10700
YS	3.3	52	100	88	108	108	77	12500
WA			93		62	67	75	11300
EB	3.6	53	100	74	75	75	60	11600
AA	1.5	34	89	61	71	80	60	17000
IG	2.1	35	80	45	48	60	49	11100
KA	1.4	31	98	35	39	40	36	10600
AO	1.5	9	63	15	32	51	23	18600
LV	2.9	11	49	15	17	35	15	15200
TB			49		15	31	15	12100
BF	0.8	2	17	3	5	27	4	14300

¹ The clearance values are not corrected to hold for 1.73 m² body surface

Table VI Series A I (Chapter VII) Clearance of Hypaque inulin and PAH with intravenous infusion technique quotient Hyp/inulin clearance and filtration fraction.

Case	Hcr %	Period		Urine flow ml/min	Clearance			Quotient Hyp Inulin	Filtration Fraction $\times 100$
		No	duration min		Hyp ml/min	Inulin ml/min	PAH ml/min		
MA	39	1	20	4.1	122	79	303	1.54	26
		2	20	2.9	118	97	376	1.22	26
		3	20	3.0	130	131	647	0.99	20
		M ¹			123	102	442	1.21	23
		M corr ²			109	91	393		
UA	31	1	20	2.2	89	116	711	0.77	16
		2	20	1.1	100	87	466	1.15	19
		3	31	1.0	100	105	542	0.95	19
		M			96	103	573	0.94	18
		M corr			103	110	613		
EO	30	1	33	3.3	73	53	232	1.38	23
		2	26	1.1	91	109	389	0.83	28
		3	26	1.4	89	89	392	1.00	23
		M			81	84	338	1.01	25
		M corr			99	93	399		
DS	37	1	25	1.0	118	100	499	1.18	20
		2	25	1.1	151	81	537	1.86	15
		3	25	0.8	145	102	483	1.42	21
		M			138	94	506	1.46	19
		M corr			106	73	388		
DJ	39	1	20	1.5	135	116	669	1.16	17
		2	20	1.5	134	128	763	1.06	17
		3	20	1.6	135	117	672	1.15	17
		M			135	120	701	1.13	17
		M corr			138	121	708		
JL	42	1	20	1.4	150	130	691	1.15	19
		2	20	3.3	167	131	820	1.27	18
		3	20	7.9	152	144	744	1.06	19
		M			156	135	752	1.16	18
		M corr			136	118	656		
VI	40	1	20	6.8	114	123	660	0.93	19
		2	20	6.3	117	76	381	1.54	20
		3	20	4.9	118	115	604	1.03	19
		M			116	105	548	1.11	19
		M corr			119	108	564		

Case	Hcr %	Period		Urine flow ml/min	Clearance			Quotient Hyp / Insulin	Filtration Fraction × 100
		No	duration min		Hyp ml/min	Insulin ml/min	PAH ml/min		
SH	39	1	20	12.9	123		587		
		2	20	10.5	117		571		
		3	20	8.7	118		524		
		M			119		561		
		M corr			108		511		
AB	41	1	20	2.7	112	110	589	1.02	19
		2	20	2.8	104	76	541	1.37	14
		3	20	3.9	139	137	637	1.01	22
		M			118	108	589	1.10	18
		M corr			105	96	524		
SP	38	1	20	1.6	136	136	594	1.00	23
		2	20	1.6	120	151	704	0.79	21
		3	20	2.1	115	140	629	0.82	22
		M			124	142	642	0.87	22
		M corr			114	130	591		
IH	39	1	20	9.0	140		457		
		2	19	10.6	114		374		
		3	19	11.1	117		415		
		M			124		415		
		M corr			126		423		
SL	35	1	20	4.7	82		505		
		2	20	3.3	95		408		
		3	20	5.6	88		503		
		M			88		472		
		M corr			80		410		
NS	41	1	25	11.0	120	69	408	1.74	17
		2	25	1.4	159	97	651	1.64	15
		3	20	2.1	145	134	614	1.08	22
		M			141	100	558	1.41	18
		M corr			117	83	464		
GL	41	1	20	14.6	133	112	701	1.19	16
		2	20	14.3	138	100	687	1.38	15
		3	20	8.6	132	107	678	1.23	16
		M			134	106	689	1.26	15
		M corr			106	84	544		

Case	Hcr %	Period		Urine flow ml/min	Clearance			Quotient Hyp / Insulin	Filtration Fraction x 100
		No	duration min		Hyp ml/min	Insulin ml/min	PAH ml/min		
EN	38	1	20	7.6	131	122		1.07	
		2	21	8.1	130	138		0.94	
		3	20	6.4	125	139		0.90	
		M			129	133		0.97	
		M corr			111	114			
BK	42	1	20	3.2	109	80	311	1.38	26
		2	20	5.8	101	87	457	1.16	19
		3	20	8.2	103	54	220	1.91	25
		M			104	74	329	1.42	22
		M corr			100	71	316		
NK	43	1	20	11.0	122	106		1.15	
		2	20	10.0	117	134		0.87	
		3	20	4.9	101	161		0.63	
		M			113	134		0.85	
		M corr			98	117			
JJ	38	1	25	1.5	154	115	494	1.34	23
		2	25	2.5	139	125	506	1.11	25
		3	25	5.5	136	96	361	1.42	27
		M			143	112	454	1.28	25
		M corr			142	111	449		

¹ Mean value of 3 clearance periods

² Mean value corrected to hold for 1.73 m² body surface

Table \ II Series A 2, 3, 4 (Chapter VII) Clearance of Hypaque with intravenous infusion technique

Case	Period		Urine flow ml/min	Hypaque clearance ml/min
	No	duration min		
GJ	1	20	1 1	153
	2	21	1 1	142
	3	20	1 0	136
	M ¹			144
	M corr			147
RE	1	21	0 6	84
	2	20	0 6	104
	3	21	1 0	104
	M			97
	M corr			114
VN	1	22	1 9	75
	2	22	4 6	88
	3	22	4 6	100
	M			91
	M corr			91
US	1	23	0 4	106
	2	26	0 4	93
	3	21	0 5	118
	M			110
	M corr			142
VH	1	22	1 2	96
	2	22	0 8	84
	3	22	0 9	89
	M			90
	M corr			96
AD	1	23	0 9	129
	2	23	0 9	126
	3	29	1 6	137
	M			131
	M corr			117
FS	1	22	0 7	96
	2	22	0 7	115
	3	22	0 5	112
	M			108
	M corr			107

Case	Period		Urine flow ml/min	Hypaque clearance ml min
	No	duration min		
MP	1	22	0.7	141
	2	22	0.8	133
	3	22	1.0	151
	M			142
	M corr			143
AP	1	22	0.7	■
	2	23	0.8	134
	3	22	0.6	116
	M			116
	M corr			120
GS	1	22	0.8	110
	2	22	0.7	133
	3	22	0.7	117
	M			120
	M corr			114
CJ	1	24	0.9	132
	2	20	0.4	90
	3	22	1.1	131
	M			110
	M corr			100

1 Mean value of 3 clearance periods

2 Mean value corrected to hold for 1.73 m² body surface

Table \ II Series A 2, 3, 4 (Chapter \ II) Clearance of Hypaque with intravenous infusion technique

Case	Period		Urine flow ml, min	Hypaque clearance ml, min
	No	duration min		
GJ	1	20	1 1	153
	2	21	1 1	142
	3	20	1 0	136
	M ¹			144
	M corr ²			147
RE	1	21	0 6	84
	2	29	0 6	104
	3	21	1 0	104
	M			97
	M corr			114
VN	1	22	1 9	75
	2	22	4 6	88
	3	22	4 6	100
	M			91
	M corr			91
LS	1	23	0 4	100
	2	26	0 4	93
	3	21	0 5	118
	M			110
	M corr			142
VH	1	22	1 2	96
	2	22	0 8	84
	3	22	0 9	89
	M			90
	M corr			96
AD	1	23	0 9	129
	2	23	0 9	126
	3	29	1 6	137
	M			131
	M corr			117
FS	1	22	0 7	96
	2	22	0 7	115
	3	22	0 5	112
	M			108
	M corr			107

Case	Her	Period		Urine Flow ml/min	Clearance			Quotient Hyp / inulin	Itra tion Frac tion ×100
		No	duration min		Hyp ml/min	Inulin ml/min	PAH ml/min		
RA	af	1	20	2.3	113	102		1.11	
		2	20	2.4	105	9		1.33	
		3	20	3.2	107	96		1.11	
		M			113	92		1.17	
		M corr			93	9			
FA		1	21	2.9	124	110	967	1.13	11
		2	21	3.6					
		3	21	1.3	88	69	496	1.42	13
		M			1.6	86	32	1.23	19
		M corr			30	3	670		
WA	37	1	20	1.9	1	60	407	1.1	10
		2	2	2	80	8	5.1	1.38	10
		3	20	2.0	6	53	518	1.43	11
		M			5	57	459	1.39	11
		M corr			88	6	84		
CA	41	1	21	5.9	86	51	268	1.69	19
		2	21	1.8	81	51	269	1.61	19
		3	21	1.2	52	63	26	1.46	21
		M			8	55	266	1.58	21
		M corr			8	1	2.1		
HU	1	1	20	5.1	5	56	255	1.41	20
		2	21	2			315	1.32	18
		3	2	2.2	5	62	462	1.2	13
		M			8	55	354	1.33	1
		M corr			82	61	5.9		
AS	44	1	21	1.7	1	48	233	1.48	21
		2	2	2.3	88	96	281	0.92	34
		3	21	2.3	1	82	213	0.87	38
		M				5	242	1.09	31
		M corr			1	69	233		
HU	44	1	0	1	62	39	249	1.59	16
		2	21	7	68	35	220	1.94	16
		3	0	4.8	63	35	192	1.81	18
		M			64	36	221	1	16
		M corr			6	38	232		

Table XIII Series II (Chapter VII) Clearance of Hypaque inulin and PAH with intravenous infusion technique quotient Hyp /inulin clearance and filtration fraction

Case	Hcr %	Period		Urine flow ml/min	Clearance			Quotient Hyp / inulin	Filtration fraction × 100
		No	duration min		Hyp ml/min	Inulin ml/min	PAH ml/min		
ÖD	39	1	20	10.1	129	90	678	1.43	13
		2	20	8.8	131	61	496	2.15	12
		3	20	4.4	118	102	763	1.16	13
		M ¹			126	84	646	1.49	13
		M corr ²			117	78	601		
BE	33	1	20	1.1	102	81		1.26	
		2	20	2.0	103	81		1.27	
		3	20	3.7	115	86		1.34	
		M			107	83		1.29	
		M corr			116	90			
IE	41	1	22	1.8	120	65	327	1.85	20
		2	22	1.8	100	65	312	1.55	21
		3	22	2.5	131	88	553	1.49	15
		M			117	73	408	1.61	18
		M corr			111	69	388		
VL	42	1	20	2.5	131	119	523	1.10	23
		2	20	3.4	131	113	579	1.16	20
		3	20	4.2	132	172	733	0.77	23
		M			131	135	612	0.98	22
		M corr			110	113	514		
AO	34	1	23	1.8	130	101		1.29	
		2	22	2.3	124	90		1.38	
		3	25	1.4	93	109		0.87	
		M			116	100		1.16	
		M corr			107	92			
BA		1	27	1.4	121	90	376	1.34	24
		2	26	2.4	103	82	348	1.26	24
		3	22	2.8	97	89	274	1.09	32
		M			107	87	333	1.23	26
		M corr			96	78	300		
JA		1	20	4.9	86	38	244	2.26	16
		2	20	5.3	93	64	378	1.45	17
		3	22	4.7	102	68	417	1.50	16
		M			94	57	346	1.65	16
		M corr			96	58	353		

Case	Hcr %	Period		Urine flow ml/min	Clearance			Quotient Hyp / Insulin	Filtration Frac- tion × 100
		No	duration min		Hyp ml/min	Insulin ml/min	PAH ml/min		
BA	34	1	20	2.5	113	102		1.11	
		2	20	2.4	105	79		1.33	
		3	20	3.2	107	96		1.14	
		M			105	92		1.17	
		M corr			93	79			
CA		1	21	2.9	121	110	967	1.13	11
		2	21	3.6					
		3	21	1.3	88	62	496	1.42	13
		M			106	86	732	1.23	12
		M corr			90	73	620		
WA	37	1	20	1.9	70	60	407	1.17	15
		2	20	2.0	80	58	571	1.38	10
		3	20	2.0	76	53	518	1.43	10
		M			75	57	493	1.32	11
		M corr			86	67	584		
CA	41	1	24	5.3	86	71	268	1.69	19
		2	21	1.8	83	51	262	1.63	19
		3	21	1.2	52	63	267	1.46	21
		M			87	55	266	1.55	21
		M corr			85	54	261		
BK	45	1	20	5.1	79	56	277	1.41	20
		2	20	2	75	57	315	1.32	18
		3	20	2.2	79	62	462	1.27	17
		M			78	58	351	1.33	17
		M corr			82	61	359		
AS	44	1	21	1.7	71	48	233	1.48	21
		2	20	2.3	88	96	281	0.92	34
		3	21	2.3	71	82	213	0.87	35
		M			77	75	242	1.02	31
		M corr			71	69	233		
RH	54	1	20	7.1	62	35	219	1.59	22
		2	24	7.7	68	35	220	1.94	16
		3	20	4.8	63	35	192	1.80	22
		M			64	36	220	1.77	16
		M corr			67	38	232		

Case	Hcr %	Period		Urine flow ml/min	Clearance			Quotient Hyp / Insulin	Filtration Fraction × 100
		No	duration min		Hyp ml/min	Insulin ml/min	PAH ml/min		
HJ	37	1	21	6.2	81	79		1.03	
		2	21	6.5	90	93		0.97	
		3	21	4.3	96	101		0.83	
		M			86	92		0.93	
		M corr			65	69			
FB	39	1	20	4.3	55	48	135	1.21	36
		2	20	2.6	59	48	124	1.23	39
		3	20	2.2	63	52	130	1.21	40
		M			60	49	130	1.22	75
		M corr			59	48	127		
AA	30	1	20	1.3	40	51	196	0.91	25
		2	20	1.9	38	55	169	1.00	31
		3	20	2.7	74	67	337	1.10	20
		M			60	60	234	1.01	26
		M corr			56	56	215		
IG	35	1	25	3.2	50	60		0.83	
		2	25	2.7	50	51		0.91	
		3	27	2.7	47	50		0.91	
		M			49	51		0.90	
		M corr			50	55			
KK	41	1	20	3.0	39	31		1.15	
		2	20	1.7	41	30		1.03	
		3	20	1.4	39	36		1.08	
		M			36	33		1.09	
		M corr			39	36			
AG	27	1	25	1.0	21	12	32	1.75	39
		2	25	1.1	27	18	43	1.50	42
		3	27	0.9	21	13	26	1.62	30
		M			23	11	31	1.60	11
		M corr			22	13	32		
LB	23	1	22	1.7	18	22		0.82	
		2	25	1.9	14	16		0.88	
		3	21	1.7	14	16		0.83	
		M			15	18		0.85	
		M corr			16	19			
LV	26	1	20	3.1	15	23		0.65	
		2	20	2.5	15	21		0.71	
		3	20	3.1	16	25		0.61	
		M			15	23		0.67	
		M corr			15	23			

1. Mean value of 3 clearance periods

Mean value corrected to hold for 1.75 m² body surface

Table XIV Series C (Chapter VII) Clearance of Hypaque and inulin with intravenous infusion technique and quotient Hyp/inulin clearance before and after injection of Probenecid

Case	Probenecid mg	Period		Urine flow ml min	Clearance		Quotient Hyp / Inulin
		No	duration min		Hyp ml min	Inulin ml min	
R1	500	1	15	90	80	72	1.11
		2	16	49	70	79	0.83
		3	16	46	71	83	0.86
		4	11	63	83	98	0.85
		5	17	43	75	77	0.97
LS	500	1	15	13.1	110	103	1.07
		2	16	29	103	107	0.96
		3	15	39	98	110	0.83
		4	15	38	91	119	0.83
		5	15	47	100	125	0.80
M	500	1	1	7.9	101	72	1.40
		2	17	4.1	76	71	1.35
		3	16	8.1	73	69	1.14
		4	16	3.2	72	78	0.92
		5	16	3.0	74	60	1.23
K6	1000	1	15	4.5	103	38	1.10
		2	1	3.7	117	112	1.05
		3	16	2.8	103	108	0.95
		4	18	1.5	90	97	0.93
		5	18	1.5	85	111	0.77
AL	1500	1	15	13.1	144	116	1.21
		2	15	9.8	117	86	1.36
		3	17	1.1	113	129	0.88
		4	16	2.3	112	135	0.83
		5	16	8.9	111	132	0.84
HR	2000	1	17	3.4	59	51	1.16
		2	16	3.5	68	55	1.25
		3	14	2.6	47	75	0.63
		4	16	1.2	50	55	0.85
		5	16	0.8	59	65	0.91
LN	2000	1	18	3.3	71	57	1.25
		2	16	3.8	75	60	1.25
		3	16	8.2	67	53	1.26
		4	17	1.0	64	36	1.78
		5	17	2.8	63	58	0.64

Case	Probenecid mg	Period		Urine flow ml/min	Clearance		Quotient Hyp / Inulin
		No	duration min		Hyp ml/min	Inulin ml/min	
LC	500	1	17	23	96	85	1.13
		2	17	23	92	76	1.21
		3	19	29	86	72	1.19
		4	19	31	79	57	1.40
		5	19	52	87	79	1.10
VW	1500	1	16	99	78	59	1.32
		2	18	91	79	58	1.36
		3	23	37	66	50	1.32
		4	17	39	67	40	1.66
		5	17	42	70	53	1.32

Table XX Series A (Chapter VIII) Renogram parameters retention serum $\frac{P}{N}$ and diagnosis in controls

Case	Sex Age (years)	Renogram						Chest curve Ret ²		P mg %		Diagnosis
		Right			Left			°.	mg %.			
		Up- take	I xer I ¹	L xer II ¹	Up- take	I xer I	L xer II					
IC	M 22	1 053	1 252	1 162	1 053	1 357	1 167	59				Healthy
UM	M 22	1 069	1 178	1 115	1 083	1 286	1 167	55				"
GO	M 22	1 097	1 238	1 130	1 080	1 169	1 143	57				"
BL	M 22	1 139	1 480	1 220	1 089	1 459	1 276	57				"
SI	M 24	1 095	1 400	1 200	1 090	1 390	1 214	46				"
BB	M 24	1 063	1 153	1 154	1 031	1 220	1 242	59				"
OO	M 25	1 037	1 217	1 150	1 100	1 263	1 226	52				"
JM	M 25	1 159	1 152	1 179	1 119	1 218	1 222					"
VI	M 26	1 108	1 191	1 175	1 093	1 273	1 151	57				"
TI	M 28	1 059	1 342	1 188	1 045	1 414	1 261	52				"
FO	M 17	1 025	1 201	1 161	1 037	1 263	1 223	55	24			Cardiac neurosis
IP	M 18	1 073	1 353	1 259	1 058	1 383	1 288	54	23			Cephalalgia
JI	M 24	1 076	1 075	1 118	1 086	1 091	1 184	48	30			Cephalalgia
BJ	M 24	1 092	1 311	1 165	1 092	1 311	1 165	50	32			Rheum. arthritis
SG	M 27	1 065	1 384	1 263	1 049	1 370	1 305	58	33			Diss sclerosis
AO	I 30	1 167	1 403	1 241	1 140	1 473	1 257	46	28			Psychoneurosis
SN	M 32	1 101	1 352	1 255	1 047	1 389	1 180	53	26			Trigeminal neuralgia
LI	M 33	1 089	1 220	1 253	1 076	1 278	1 200	65	29			Icteric reactions
SA	M 33	1 063	1 473	1 250	1 038	1 367	1 167	47	30			Gastric ulcer
SZ	M 34	1 108	1 449	1 216	1 074	1 338	1 213	60	33			Cephalalgia
AB	M 36	1 059	1 130	1 184	1 056	1 238	1 121	57	32			Duodenal ulcer
IO	I 39	1 051	1 362	1 184	1 069	1 329	1 197	56	28			Hyper-γ globulinem
UI	M 40	1 073	1 313	1 143	1 051	1 271	1 107	66	25			Gastric ulcer
MM	M 42	1 082	1 413	1 150	1 038	1 316	1 169	53	29			Acute gastritis
OB	M 43	1 034	1 191	1 099	1 049	1 270	1 125	60	26			Cephalalgia
IV	M 48	1 091	1 123	1 204	1 055	1 233	1 154	57	32			Cholelithiasis
HB	M 49	1 091	1 160	1 186	1 061	1 226	1 154	61	34			Lumbar disc degen.
ZH	I 50	1 055	1 265	1 360	1 036	1 246	1 326	56	31			Cholelithiasis
II	M 55	1 117	1 227	1 158	1 070	1 221	1 160	49	29			Duodenal ulcer
IK	M 57	1 042	1 350	1 266	1 087	1 444	1 321	66	24			Epilepsy
IN	M 7	1 057	1 202	1 167	1 065	1 192	1 099	67	31			Cephalalgia

¹ Excretion ratio 15/30² Excretion ratio 5/15³ Retention per cent

Table XVI Reproducibility of renograms in 10 controls Two investigations (A and B) at 11 day interval

Case	Renogram								Chest curve Ret °	
	Right				Left					
	Uptake		I over I		Uptake		I over I			
	A	B	A	B	A	B	A	B		
LL	1 042	1 031	1 266	1 316	1 087	1 099	1 321	1 377	66	53
LP	1 073	1 119	1 259	1 201	1 058	1 122	1 248	1 224	54	60
SN	1 101	1 035	1 255	1 196	1 087	1 073	1 150	1 231	53	55
IL	1 117	1 100	1 158	1 193	1 070	1 067	1 160	1 176	49	53
SZ	1 108	1 153	1 216	1 153	1 074	1 114	1 213	1 208	60	54
BI	1 139	1 076	1 220	1 189	1 089	1 078	1 276	1 268	57	51
OB	1 034	0 941	1 099	1 122	1 089	1 073	1 125	1 151	60	56
LI	1 089	1 067	1 253	1 239	1 076	1 015	1 200	1 238	61	61
IO	1 051	1 000	1 184	1 171	1 069	1 057	1 197	1 150	56	67
SG	1 065	1 083	1 263	1 197	1 019	1 093	1 305	1 222	58	57
Mean	1 082	1 065	1 217	1 198	1 075	1 079	1 227	1 225	58	56

Table XVII Series B—G (Chapter VIII) Renogram parameters, retention and laboratory findings in cases of renal parenchymal diseases hypertension post nephrectomy and aplasia

Series B Chronic glom. nephritis

Case	Sex Age (years)	Renogram				Chest curve Ret	Laboratory findings			
		Right		Left			N P N	Serum creati nine	(creati nine clea rance ^a ml min	Max conc
		Up take	I ver I ¹	Up take	I ver I					
						%	mg %	mg %		g ml
RS	F 47	0.931	1.101	0.971	1.121	70	41	1.00	123	1.016
TJ	M 33	0.923	1.029	0.956	1.021	68	33	1.11	89	1.015
SL	F 49	0.909	1.080	0.923	1.105	71	31	1.42	51	1.015
MI	F 44	0.951	1.157	0.964	1.118	55	46	1.11	12	1.021
AS	F 30	0.956	1.079	0.922	1.071	78	155	4.05	11	
VB	F 43		1.067		1.013	81	58	6.22	12	
BI	M 37	0.970	1.016	1.000	1.016	88	212	10.50	9	
GI	M 30	0.990	1.011	1.000	1.014	79	166	17.70		

Series C Chronic pyelonephritis

Case	Sex Age (years)	Renogram				Chest curve Ref ²	Laboratory findings			
		Right		Left			V P V	Serum creati nine	Creati nine clearance ³ ml min	Max conc
		Up take	I xer I ¹	Up take	I xer I					
						%	mg %	mg %		g ml
KS	1 19	1 013	1 063	1 105	1 000	65	25	0 66	133	
BM	1 46	0 968	1 167	1 089	1 321	57	32	0 82	112	1 026
VI	1 56	1 014	0 994	1 069	1 136	63	25	0 82	109	1 025
BT	1 41	0 923	1 105	1 037	1 118	58	26	0 94	90	1 028
AT	1 52	0 968	1 083	1 000	1 109	71	34	1 28	65	1 026
RQ	1 52	1 000	1 026	0 976	1 094	69	23	1 33	59	1 012
MH	1 37	1 000	1 028	1 053	1 088	69	42	1 81	56	1 012
WB	1 54	0 915	1 065	0 953	1 061	72	35	1 74	43	
HH	1 52	0 944	1 036	0 918	1 025	83	78	2 07	35	
EP	1 61	0 939	1 033	0 935	1 031	75	48	2 40	35	
AO	M 55	0 967	1 010	1 020	1 033	72	50	2 89	30	
OA	M 64	1 000	1 091	1 000	1 057	64	60	2 81	19	
TB	M 65	0 951	1 031	0 976	1 056	75	69	4 00	23	1 009
MW	1 50	0 951	1 026	0 964	1 044	84	90	4 12		
AL	1 51	0 945	1 032	0 937	1 026	84	78	4 14	15	
SB	1 48	0 916	1 016	0 964	1 020		118	6 09	10	
SJ	1 36	0 937	1 010	0 961	1 070	69	142	11 00	9	1 007
LI	1 29	0 900	1 031		1 025	91	162			

Series D Idiopathic disease

LI	M 21	1 160	1 107	1 000	1 075	60	32	0 96	143	1 010
PN	1 44	1 088	1 016	0 950	1 015	82	11	1 39	72	
KL	M 37		1 029	1 000	1 000	70		3 31	38	1 009
VC	M 38	1 000	1 055	1 000	1 058	78	199	12 00	5	

Series E Other parenchymal diseases

TY	M 54	1 026	1 155	1 053	1 098	62	30	0 80	153	1 027
HS	M 33	1 083	1 100	1 071	1 111	56	24	0 69	148	1 022
MI	M 21	1 038	1 149	1 019	1 163	62	28	0 96	137	1 028
BN	M 55	1 085	1 110	1 075	1 162	60	41	0 82		
HI	1 42	0 945	1 075	1 141	1 241	66	21	0 53	131	
BN	M 2	1 086	1 210	1 018	1 324	64	32	1 08	110	1 045
AO	M 53	0 980	1 080	1 088	1 094	62	32	0 39	77	1 022
FM	M 54	0 985	1 115	1 111	1 122	57	44	1 10	93	1 018
FN	1 19	1 108	1 160	1 074	1 234	72	25	0 76	83	1 023
HI	M 29	1 050	1 058	0 955	1 089	71	46	1 13	80	1 016

Table XVI Reproducibility of renograms in 10 controls Two investigations (A and B) at 2 day interval

Case	Renogram								Chest curve Ret %	
	Right				Left					
	Uptake		I xcr I		Uptake		I xcr I		A	B
	A	B	A	B	A	B	A	B		
EL	1 042	1 031	1 266	1 316	1 087	1 099	1 324	1 373	60	53
LP	1 073	1 118	1 259	1 204	1 058	1 122	1 285	1 224	51	60
SN	1 101	1 085	1 255	1 196	1 087	1 073	1 180	1 231	53	55
IL	1 117	1 100	1 158	1 193	1 070	1 063	1 160	1 176	19	53
SZ	1 106	1 153	1 216	1 153	1 074	1 111	1 213	1 208	60	54
BI	1 139	1 076	1 220	1 189	1 089	1 078	1 276	1 266	57	51
OB	1 034	0 941	1 099	1 122	1 089	1 073	1 125	1 154	60	56
IT	1 089	1 067	1 253	1 239	1 076	1 015	1 200	1 238	67	61
IO	1 051	1 000	1 181	1 173	1 069	1 057	1 197	1 150	56	63
SC	1 065	1 083	1 263	1 197	1 049	1 093	1 305	1 222	58	57
Mean	1 082	1 065	1 217	1 198	1 075	1 079	1 227	1 225	58	56

Table XVII Series B—G (Chapter VIII) Renogram parameters retention and laboratory findings in cases of renal parenchymal diseases hypertension post nephrectomy and aplasia

Series B Chronic glom nephritis

Case	Sex Age (years)	Renogram				Chest curve Ret %	Laboratory findings			
		Right		Left			N P N	Serum creati nine	Creati nine clearance ^a	Max conc
		Up take	I xcr I	Up take	I xcr I					
						%	mg %	mg %	ml min	g ml
RS	1 47	0 934	1 101	0 971	1 121	70	41	1 00	123	1 016
TJ	VI 33	0 923	1 029	0 956	1 021	68	33	1 15	89	1 019
SE	1 49	0 909	1 080	0 923	1 105	71	34	1 12	51	1 01
VI	1 44	0 951	1 157	0 964	1 118	85	46	1 11	12	1 021
AS	1 30	0 956	1 079	0 922	1 071	78	155	1 05	13	
VB	1 43		1 067		1 013	81	88	6 22	12	
BI	VI 37	0 970	1 016	1 000	1 016	88	212	1050	9	
CL	VI 30	0 990	1 011	1 000	1 014	79	166	13 70		

Table VIII Series II (Chapter VIII) Renogram parameters and diagnosis in cases with acute ureteral obstruction (suspect or proven)

Case	Sex Age (years)	Renogram				Diagnosis
		Right		Left		
		Lp take	Excr II ¹	Lp take	Excr II	
ML	F 35	1 000	1 068	1 000	1 068	Acute cystitis
KL	M 46	1 047	1 075	1 065	1 116	Duodenal ulcer
BS	F 19	1 167	1 203	1 100	1 212	Acute cystitis
IP	F 62	1 022	1 178	0 989	1 193	Acute cholecystitis
AN	M 44	1 064	1 143	1 050	1 083	Ischemic lumbago
AG	F 28	1 000	1 250	1 000	1 320	Acute cholecystitis
KH	M 41	1 011	1 143	1 036	1 203	Abdominal pain
WH	F 38	0 904	1 079	0 914	1 108	Acute urethritis
NP	F 72	1 056	1 327	1 063	1 357	Diab. mellitus + Herpes Zoster
ML	F 40	1 063	1 015	1 000	1 000	Acute pancreatitis
BP	F 14	1 057	1 310	1 038	1 370	Acute cystopyelitis
DA	M 25	1 095	1 132	1 059	1 200	After passage of stone
LI	F 39	1 072	1 063	1 093	1 146	Right ov. cyst.
ML	F 56	0 943	1 119	1 140	1 297	Right renal aplasia
AS	M 33	1 081	0 870	1 103	1 138	Lecterolithiasis + Obstruction
FI	M 35	1 036	0 688	1 064	1 132	" "
BR	M 28	1 032	0 925	1 021	1 184	" "
BW	M 52	1 026	0 919	1 050	1 261	" "
CS	M 52	1 061	0 916	1 020	0 912	" "
FL	M 0	1 063	0 991	1 022	1 250	" "
CC	M 41	1 145	0 718	1 111	1 087	" "
SO	M 42	1 035	0 858	1 018	1 174	" "
TA	M 40	1 057	0 786	1 079	1 388	" "
CR	M 49	1 045	0 815	1 083	1 294	" "
FR	F 50	1 031	0 886	0 954	1 163	Obstruction
AB	F 21	1 119	0 863	1 049		"
OR	F 50	1 023	0 934	1 023	1 262	"
CB	M 59	1 158	0 580	1 136	1 217	"
NW	M 40	0 955	1 196	1 053	0 909	Lecterolithiasis + Silent kidney
BO	M 25	1 051	0 851	1 074	1 241	"
NM	M 70	1 085	1 097	1 071	0 918	Silent kidney
MI	M 48	1 061	0 842	1 057	1 149	Obstruction
LO	M 49	1 047	1 222	1 017	1 294	Obstruction(?)

¹ Excretion ratio 5:15

Case	Sex Age (years)	Renogram				Chest curve Ret ²	Laboratory findings			
		Right		Left			N P N	Serum creati nine	Creati nine clea rance ³	Max conc
		Up take	I xcr I ¹	Up take	I xcr I					
RM	M 54	1 059	1 068	1 032	1 016	65	35	0 85		
EB	F 54	0 992	1 075	0 982	1 081	73	48	1 17	45	1 010
CN	F 22	1 033	1 171	1 071	1 214	72	49	1 27		
AA	M 60	0 979	1 027	0 979	1 000	68	37	1 44	62	
KK	F 29	0 938	1 122		1 129	82	37	1 73	37	1 014
IP	M 52	0 953	1 043	0 933	1 091	92	38	2 13	42	1 008
EF	F 42	0 900	1 031	0 943	1 033	87	124	2 02	12	
CA	M 34	0 913	1 034	0 936	1 016	81	122			

Series F Hypertension

BF	F 34	0 908	1 111	1 099	1 361	64	26	0 76	129	1 026
EJ	F 41	1 019	1 013	1 100	1 118	67	30	0 83	117	
YS	M 40	1 000	1 075	0 969	1 041	58	40	1 12	87	1 023
NN	F 51	1 137	1 182	1 150	1 230	67	37	0 93	96	
ON	F 52	1 025	1 168	1 023	1 119	63	25	0 76	83	
AH	F 45	1 000	1 000	0 984	1 036	90	66	1 71	51	
AN	M 56	0 971	1 111	0 985	1 115	68	38	1 76	54	
IG	F 36	1 037	1 013	1 047	1 069	73	36	1 33	43	1 017
JG	M 46	1 037	1 019	1 036	1 019	70	34	1 72	41	
AL	M 67	0 837	1 067	0 850	1 032	77	104	3 10	27	

Series C Nephrectomy or aplasia

LA	F 17	0 813	1 063	1 255	1 290	59	33			
TO	F 41	0 897	1 066	1 070	1 172	63	26	1 18	90	1 016
ES	M 59	1 210	1 259	0 934	1 042	68	33	1 13	17	
WA	F 64	0 868	1 068	1 070	1 113	66	40	0 82	131	
HZ	F 64	1 070	1 112	0 928	1 119	74	38	1 23	49	1 011
OS	M 40	1 179	1 113	0 917	1 079	54	32			1 021

¹ Excretion ratio 15/30

² Retention per cent

³ Corrected to hold for 1.73 m² body surface

Series C Chronic pyelonephritis

Case

KS	Lr	R: plain p. calyces and pelvis. Parenchymal condens. over spine L: as right. Horseshoe kidneys.
BM	Lr	R: deformed with depression of upper calyx group. Small (10 x 5 cm) L: normal excret. and morph. (13.5 x 7.4)
	Ang	R: normal renal art. Small kidney
VL	Lr	R: deformed renal pelvis. Normal excret. L: normal excret. and morph.
	Iyel	R: double renal pelvis and ureter. Deformed pelvis
WZ	Lr	R: fragmenting (11 x 6) L: normal
	Iyel	R: all calyces deformed. All calyx groups filling of pealized spaces
AT	Lr	R and L: normal
UB	Lr	R and L: normal.
	Aut	Chronic pyelonephr. with bilateral contraction. R 30 g, L 60 g. Renal art. narrow with scler. plaque
III	Lr	R and L: poor excret. Morph. not evaluable
	Aut	Chronic pyelonephr. Small kidneys. R 30 g, L 110 g
II	Lr	R and L: poor excret. Calyces and pelvis dilated (Hydronephrosis). Bilateral ureter. catheterization produced no relief. No signs of ureter stone
AO	Lr	R and L: poor excret. Bilateral renal pelvis. Multiple concretions. Bilateral catheterization of ureter unsuccessful
OA	Lr	R and L: poor excret. Morph. not evaluable
	Iyel	Broadened pelvis. Several cavities filled with contrast medium
	Lap	Left nephrectomy
	Aut	Chronic pyelonephr. with contraction and papillary necrosis
TH	Aut	Chronic pyelonephr. L 100 g, R 110 g
MW	Lap	Previous right nephrotomy. Cross clanges resembling chronic pyelonephr.
AL	Aut	Chronic pyelonephr. with renal contraction. R 37 g, L 42 g.
SB	Aut	Bilateral chronic pyelonephr. with extensive calc. papillary necroses. R 40 g, L 45 g
SI	Aut	Bilateral chronic pyelonephr. with marked contraction. Hypertrophic and hypoplastic. L 1 g, L 65 g
II	Aut	Chronic pyelonephr. with marked papillary changes with contraction. Bilateral moderate hydronephr. and bilateral hydronephroses

Series D Polycystic disease

Case

KA	Lr	R: normal
	Aut	1 large irregular lat. outpouching and calyx group displaced
		R: aberrant renal artery, no stenosis
		1 bilateral nephrocalic effect
	Lap	1 kidneys full of cysts. Longen lat. multiloc. cystic L. kidney
AN	Aut	1 enlarged kidneys with irregular outpouchings
	Aut	1 enlarged kidneys with multiple cysts. R 460 g, L 340 g.
II	Aut	1 enlarged kidneys with irregular outpouchings
	Aut	1 enlarged kidneys with cysts of various sizes.

Table XX Series I (Chapter VIII) Renogram parameters retention and laboratory findings in cases of hydronephrosis

Case	Sex Age (years)	Renogram				Chest curve Ret ² %	Laboratory findings			
		Right		Left			N P N	Serum creati nine mg %	Creati nine clea rance ³ ml min	Max conc g ml
		Up take	Excr I ¹	Up take	Excr I					
ÖP	M 33	1 167	1 074	1 158	1 281	57	27	0 68	144	1 023
IW	M 52	1 084	1 056	1 044	1 099	56	33	0 92	108	1 019
VP	M 45	1 087	1 106	1 108	0 886	50	35			
JW	M 57	1 011	1 035	1 115	1 136	70	32	0 84	100	1 021
DD	I 50	1 167	0 954	1 106	1 105	58	38			1 022
LL	I 33	1 014	0 968	1 031	1 088	67	26	0 96		
AM	M 55	1 062	1 311	1 038	0 957	66	31			1 011
KO	M 49	1 102	1 135	1 087	0 862	54	38	0 88	111	1 010
SS	I 50	1 077	0 978	1 077	1 111	63	36	0 63	91	1 026
SA	I 57	1 123	1 000	1 000	0 971	63		0 93	94	
LH	M 69	0 868	1 092	1 104	1 090	67	18	1 27	57	
FJ	M 69	0 903	1 054	1 098	0 961	58	41			1 011
IN	I 57	0 947	1 020	1 054	0 667		31	0 90	113	

¹ Excretion ratio 15/30² Retention per cent³ Corrected to hold for 1.73 m² body surfaceTable XX Series B—I (Chapter VIII) Results of roentgen examination laparotomy and autopsy
Abbreviations R Right kidney L Left kidney Plain Plain roentgenography Ur Excretion
urography Pyel Retrograde pyelography Ang Selective renal angiography Aort Aortography
Lap Laparotomy Aut Autopsy

Series B Chronic glom nephritis

Case		
RS	Aut	Subchron glom nephrit with contraction R 110 g L 120 g (Bilat 11 50)
TJ	Ur	R poor excr Morph not evaluable (12×5) L poor excr Morph not evaluable (11×5)
SL	Aut	Chron glom nephrit with contraction R 80 g L 70 g
	Ur	R fragmentary filling Morph not evaluable (10×50) L fragmentary filling Morph not evaluable (11×50)
MI	Aut	Chron glom nephrit with bilat contraction R 65 g L 75 g
VB	Aut	Chron glom nephrit with bilat contraction R 60 g L 60 g
BI	Aut	Chron glom nephrit with bilat contraction R 75 g L 85 g
GL	Aut	Chron glom nephrit with bilat contraction R 80 g L 100 g
		Small plaque fl in ren art Stenosis (4 cm) at origin of L ren art — plaque

XX	Ur	R and L normal
OX	Lr	R normal (12.5×6) L normal (12×4)
	Aut	Nephrosclerosis Ren art normal Both kidneys small R 10.5 ■ L 8.0 g.
XH	Lr	R poor excr Morph unevaluable Small (11×6) L poor excr Morph unevaluable (12.5×5)
YN	Lr	R normal excr and morph Small (11.2×4) L normal excr and morph Small (10.7×4.5)
IG	Lr	R and L normal
JG	Lr	R and L normal
	Aut	Nephrosclerosis R 110 g L 100 g Ren art normal dilat

Series C Nephrectomy upstasis

Case

LA	Lr	R no excr No condens L normal excr I enlarged (17×17)
	Aut	R no excr No condens L normal
TO	Lr	R no excr No condens L normal excr and shape enlarged
	Uret	Catheter could be passed only 3—4 cm up right ureter
	Aut	(1 years after test) R no ren tissue demonstrable I chron and acute pyelonephritis
LS	Lap	Previous L nephrectomy Chron pyelonephritis
WA	Lap	Previous R nephrectomy Chron pyelonephritis with nephroblastosis
WF	Lap	Previous I nephrectomy I local interstit nephritis
OS	Lap	Previous I nephrectomy Chron pyelonephritis with nephroblastosis

Series H Acute ureteral obstruction

Case

VS	Lr	R no excr but condens in parenchyma I inhead coner in ureteric orifice Obstruction with coner
		I no coner Normal excr
LI	Lr	R no excr Coner 10×8 mm at level of L II I no coner Normal excr
	Lap	C retrolithotomy
DB	Lr	R obstruction and dilat pelv and uret. filled down to coner in lower ureter Obstruction with coner
		L no coner Normal excr
RW	Lr	R after 12 min filling of moderately dilat pelv + ureter down to small in transural coner in ureter Obstruction with demonstrable coner
		I no coner Normal excr
UN	Lr	R no coner Normal excr I no excr after 30 min only condens in parenchyma Small coner at L IV Obstruction with ureter coner
LI	Lr	R retarded excr filling of mod dilat ren pelv Ureter dilat down to coner at I II Obstruction with coner
		L no coner Normal excr

VG	<i>Plain</i>	Enlarged kidneys with irregular outlines Abundant calcifications
	<i>Aut</i>	Very large cystic kidneys Pelvic deform and distended calyces R 3030 g L 2760 g

Series E Other parenchymal diseases

Case

TY	<i>Ur</i>	R normal L double pelv and ureter (16×7.5)
HS	<i>Ur</i>	R normal excr L normal excr Numerous small papill calculi in both kidneys
MI	<i>Ur</i>	R and L normal
RA	<i>Ur</i>	R normal excr and morph (13×7.5) L normal excr and morph (16×8)
	<i>Port</i>	Bilat normal Normal renal arteries L kidney larger than R
	<i>Aut</i>	R 200 g (13.5×6.5) Lower pole contains small coner and shows slight pye lonephritis Normal cortical and medullary zone L 100 g (11.5×6)
HE	<i>Ur</i>	R thin filling Morph not evaluable Small kidney L normal
	<i>Port</i>	R small ren art Small kidney (6×2) L normal (15.6×7.5)
	<i>Lap</i>	Nephrectomy Hypoplastic kidney Chron pyelonephr
BA	<i>Ur</i>	R and L normal
AO	<i>Ur</i>	R and L normal
KM	<i>Ur</i>	R normal excr Small (12.5×5) Double pelv L normal excr and morph (14.5×7)
	<i>Ang</i>	R normal ren art
RM	<i>Ur</i>	R normal L retarded filling of large plump calyces
	<i>Pyel</i>	L plump, large calyces
	<i>Lap</i>	Nephrectomy Widespread tb and large cavern in lower pole Caseous necrosis
AV	<i>Ur</i>	Bilat horseshoe kidneys displaced to left Excr slightly retarded and thin No coner
KK	<i>Ur</i>	R and L Bilat low condensation Bilat malrotation Double ren pelvis and ureter
	<i>Port</i>	Malrotation + anomalous ren art bilat
IP	<i>Ur</i>	R poor excr Morph not evaluable (11×5.5) L poor excr Morph not evaluable
LI	<i>Aut</i>	Chron and acute pyelonephr lesions and lesions of Kimmelstiel Wilson type R 290 g L 250 g

Series F Hypertension

Case

BL	<i>Ur</i>	R small but of normal shape (11×5) L compens hypertrophy normal shape (15×7)
	<i>Ang</i>	R narrow ren art without certain stenosis
LJ	<i>Ur</i>	R fragmentary filling of pelvis and calyces Small (11×5) L compens hypertrophy normal morph (11×7.5)
YS	<i>Ur</i>	R and L normal

		L. normal
I	Lr	R. normal
		L. signs of hydronephrosis
Ijel		R. marked dilat. of pelvis and calyces. Pelvic junction from anterior aspect of pelvis
	Lap	Verified hydronephrosis with ureteric stricture
W	Lr	R. retarded excret. Mature kidney occupied by dilat. pelvis and dilat. calyces and collecting ducts. (13.5 x 9)
		L. normal
	Lap and Aut	Large hydronephrotic sac. Ren. cancer (squamous epithelial). Chron. pyelo-perinephrit.
DD	Lr	R. retarded excret. and defect filling of pelvis and ureter. Concr. in lower ureter (in cystocele) and same concr. in ureter widening
		L. normal
	Lap	R. Lithicotomy
I	Lr	R. retarded excret. and defect filling of pelvis with dilat. calyces. No filling of ureter. Bladder deformed by extravascular lesion (abscess)
		L. normal
	Aut	(6 months later) Widespread metastases and growth over both ureters. Bilat. obstruction and signs of hydronephrosis
VI	Lr	R. normal
		L. retarded excret. filling of widened pelvis. Caudal part contained concr. Ureter springs from upper part of pelvis. Obstruction at the ureteropelvic junction
HO	Lr	R. normal
		L. retarded excret. Large kidney with dilat. pelvis and calyces. Three concr. in lower pole near caudal calyx group
	Lap	Nephrectomy (L). Hydronephrosis and chron. pyelonephritis and nephrothiasis
SS	Lr	R. retarded excret. Calyces and pelvis and collect. tubules slightly wider than normal. Ureter narrow at origin (14.5 x 6.5)
		L. normal excret. but somewhat wide and plump calyces and pelvis (16 x 6.5)
SA	Lr	Bilat. retarded excret. Dilat. of pelvis and calyces. Drainage obstructed
III	Lr	R. silent (6 x 3)
		L. slightly enlarged (14.8 x 6.4). Retarded excret. Sac-like pelvis and plump calyces. Dilat. of ureter
	Vj	An artery 2 mm wide running to aplastic kidney. No filling.
	Lj	Interstitial type of pelvis and ureteral stricture
FI	Lr	R. silent
		L. retarded excret. and defect filling of dilat. pelvis and calyces
	Aut	Interstitial cancer. R. kidneys small and atrophic (30 g). Stenosis of R. ureter. Bilat. pelvis and ureter
		L. kidneys weight 110 g. Dilat. pelvis and ureter. Narrowing of ureter at orifice
IN	L	R. resected
		L. Interstitial with marked dilat. of pelvis and narrowing of ureter and pelvis
	Lyt	L. cystic hydronephrosis

GG	Ur	R coner at level of process of I III Right ren obstruction and dilat of pelv and upper ureter No excr Caudal small coner Obstruction with demonstrable coner L no coner Normal excr
SO	Ur	R excr retarded and less than on L side Coner in lower II ureter Obstruction with coner L no coner Normal excr
FA	Ur	R retarded excr Dilat pelv and ureter Coner in lower ureter Obstruction with ureter coner I no coner Normal excr
GR	Ur	R retarded excr Dilat pelv, calyces and ureter Coner in lower ureter Obstruction with ureter coner L no coner Normal excr
LR	Ur	R slight enlargement No coner No excr but condens in parenchyma Signs of obstruction without demonstr coner L no coner Normal excr
AB	Ur	R no coner Entire ureter filled and wider than left Contrast persisted in lower ureter after exam Signs of obstruction without demonstr coner L no coner Normal excr
OR	Ur	R no coner Excr normal but slower than on other side Dilat pelv and ureter down to bladder Signs of obstruction without demonstr coner L no coner Normal excr
GB	Ur	R no coner Retarded excr Dilat calyces pelv and ureter Signs of obstruction without demonstrable coner L no coner Normal excr
NW	Ur	R no coner Normal excr L no excr Slight condens in parenchyma Coner at level of I I Obstruction with coner
BO	Ur	R slight excr Pelv and visible part of ureter dilat Small coner in ureter orifice Obstruction with coner L no coner Normal excr
NV	Lr	R no coner Normal excr Upper calyx group slightly displaced (tumour) L kidney not visualized
	Aut	(18 months after exam) Left sided nephrolithiasis Small adenoma in cortex of right kidney
AL	Lr	R no coner Excr retarded and only scanty contrast medium in calyces and pelvis Signs of obstruction without demonstr coner L no coner Normal excr
UÖ	Lr	R excr normal Ureter slightly wider than left No signs of obstruction L no coner Normal excr
	Lr	(3 weeks after exam) Right sided obstruction Coner in lower II ureter with dilat pelv and calyces ureter

In all other cases urography showed no concretum and normal excretion

Series I Hydronephrosis

Case

ÖP	Lr	R retarded excr and widened pelvis and all calyx groups L normal
	Lap	Retroperitoneal abscess on II side
IW	Lr	R retarded excr Widened pelvis and ureter down to stenosis of last 1 cm of ureter

	L normal
Lr	R normal
	L signs of hydronephrosis
Iyel	R marked dilat of pelvis and calyces 1 elvic junction from anterior aspect of pelvis
Lap	Verified hydronephrosis with ureteric stricture
Lr	R retarded excret Latire kidney occupied by dilat pelvis and dilat calyces and collecting ducts (15.5x9)
	L normal
Lap and Aut	Large hydronephrotic sac Ren cancer (squamous epithelial) Chron pyelo perinephrit
Lr	R retarded excret and defect filling of pelvis and ureter Concr in lower ureter (in cystocele) and one concr in ureter widening
	L normal
Lap	R Lithectomy
Lr	R retarded excret and defect filling of pelvis with dilated calyces No filling of ureter Bladder deformed by extravascular lesion (abscess)
	L normal
Aut	(6 months later) Widespread metastases and growth over both ureters Bilal obstruction and signs of hydronephrosis
Lr	R normal
	L retarded excret filling of widened pelvis Caudal part contained concr Ureter springs from upper part of pelvis Obstruction at the ureteropelvic junction
Lr	R normal
	L retarded excret Large kidney with dilat pelvis and calyces Three concr in lower pole near caudal calyx group
Lap	Nephrectomy (1) hydronephrosis and chron pyelonephritis and nephrolithiasis
Lr	R retarded excret Calyces and pelvis and collect tubules slightly wider than normal Ureter narrow at origin (14.5x6.5)
	L normal excret but somewhat wide and plump calyces and pelvis (16x6.5)
Lr	Bilal retarded excret Dilat of pelvis and calyces Drainage obstructed
Lr	R silent (6x3)
	L slightly enlarged (14.8x6.4) Retarded excret Sac like pelvis and plump calyces Dilat of ureter
Ang	An artery 2 mm wide running to aplastic kidney No filling
Lap	Prostat hypertrophy and ureteral stricture
Lr	R silent
	L retarded excret and defect filling of dilat pelvis and calyces
Aut	Prostat cancer Rt kidney small and atrophic (50 g) Stenosis of Rt ureter Bilal pelvis and ureter
	L kidney weight 150 g Dilat pelvis and ureter Narrowing of ureter at orifice
Lr	R nephrectomy
	L hydronephrotic with marked dilat of pelvis and narrowing of uret and pelvis
Iyel	L verified hydronephrosis

CG	Ur	R coner at level of process of L III Right ren obstruction and dilat of pelv and upper ureter No excr Caudal small coner Obstruction with demonstrable coner L no coner Normal excr
SO	Ur	R excr retarded and less than on I side Coner in lower R ureter Obstruction with coner L no coner Normal excr
TA	Ur	R retarded excr Dilat pelv and ureter Coner in lower ureter Obstruction with ureter coner L no coner Normal excr
GR	Ur	R retarded excr Dilat pelv, calyces and ureter Coner in lower ureter Obstruction with ureter coner L no coner Normal excr
LR	Ur	R slight enlargement No coner No excr but condus in parenchyma Signs of obstruction without demonstr coner L no coner Normal excr
AB	Lr	R no coner Entire ureter filled and wider than left Contrast persisted in lower ureter after exam Signs of obstruction without demonstr coner I no coner Normal excr
OR	Ur	R no coner Excr normal but slower than on other side Dilat pelv and ureter down to bladder Signs of obstruction without demonstr coner L no coner Normal excr
GB	Lr	R no coner Retarded excr Dilat calyces pelv and ureter Signs of obstruction without demonstrable coner I no coner Normal excr
VW	Lr	R no coner Normal excr L no excr Slight condens in parenchyma Coner at level of L I Obstruction with coner
BO	Ur	R slight excr Pelv and visible part of ureter dilat Small coner in urter orifice Obstruction with coner L no coner Normal excr
VM	Lr	R no coner Normal excr Upper calyx group slightly displaced (tumour) L kidney not visualized
	Aut	(18 months after exam) I left sided nephrolithiasis Small adenoma in cortex of right kidney
AL	Ur	R no coner L excr retarded and only scanty contrast medium in calyces and pelvis Signs of obstruction without demonstr coner L no coner Normal excr
UÜ	Ur	R excr normal Ureter slightly wider than left No signs of obstruction L no coner Normal excr
	Lr	(3 weeks after exam) Right sided obstruction Coner in lower R ureter with dilat pelv and calyces ureter

In all other cases urography showed no concrement and normal excretion

Series I Hydronephrosis

Case

OP	Lr	R retarded excr and widened pelvis and all calyx groups L normal
	Lap	Retroperitoneal abscess on II side
YW	Lr	R retarded excr Widened pelvis and ureter down to stenosis of last 4 cm of ureter

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Cases with renal disease in Chapter VIII (series B—C and I) The case numbers correspond to the following numbers in hospital records

Medical Department

RS	231/61	NN	613/58	JW	3315/59
TJ	2062/59	ON	1859/62	AM	2137/60
SE	2341/63	WH	1718/60	SS	2256 62
MI	3056 60	AN	2772/61		
AS	1471/58	IG	2536/62		
VB	1907/61	JG	2698/61		
BT	302 60	AL	685/58		
GE	2832 60	TY	3238/59		
KS	3040/59	HS	3308/63		
BM	3105/59	VI	1063 60		
VL	133/61	RA	1249/61		
BZ	3274/58	HE	138 61		
AT	918 61	BA	1144/60		
MH	3428 60	AO	197 61		
IB	1539 63	KM	3659 60		
HH	819/58	EA	3354/58		
LP	992 63	IJJ	399 61		
AO	2734/62	EB	1326 61		
OA	1342 62	GN	2390 64		
FB	1452 61	AA	780 60		
AU	424/62	KK	3066 63		
SB	606 60	IP	2996 64		
SJ	164/59	EF	2566/59		
LL	1113/58	EV	415 61		
KP	1448/58	TO	41 64		
ZN	134/60	LS	2017 63		
KT	1975 61	WA	1539 64		
VG	497/58	HZ	813/58		
BE	647 60	OS	3161 60		
LJ	3553/59	OP	727 58		
YS	331/64	IW	3355 60		

Surgery Department

RM	3177/58
LA	4343/57
VP	718/58
DD	884/58
KO	4529/58
LII	4428/60
FJ	960/58
IN	3310/59

*Department for
Infectious diseases*

MW	1919 60
RQ	2093/58

Department of gyn

EC	3338 60
SA	2895/60

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ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 444

JAUNDICE DURING PREGNANCY

WITH SPECIAL EMPHASIS ON
RECURRENT JAUNDICE DURING PREGNANCY
AND ITS DIFFERENTIAL DIAGNOSIS

BY

URS PETER HÄNNLÉ

ACCOMPANIES VOL 179

STOCKHOLM 1966

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FOREWORD

During the years 1959 to 1964 we had the occasion to observe personally 5 patients with recurrent intrahepatic cholestasis of pregnancy and to follow them through multiple gestations. These cases, together with a sixth case discovered in the hospital files, have been described in detail elsewhere (Haemmerli and Wyss). The perusal of the literature during the preparation of that manuscript soon revealed, that not all cases of recurrent jaundice during pregnancy could be due to recurrent jaundice of pregnancy, i.e. the cholestatic form. It also became apparent that an attempt at a differential diagnosis of recurrent jaundice during pregnancy has never been made and that many conflicting statements in the literature on recurrent jaundice during pregnancy originate in the lack of clear definitions.

The present paper attempts to fill this need. In order to achieve our purpose it has become necessary to present in a first part a brief review of the changes in so called liver function tests during uncomplicated pregnancy and in a second part a general review of jaundice during pregnancy. The third and main

part will be devoted to a description and definition of recurrent intrahepatic cholestasis of pregnancy on the basis of all verified cases in the world literature including our own patients and to a description of all other disorders which may present as recurrent jaundice during pregnancy. We believe that the literature in the third part is as complete as it possibly can be. The first two parts had to be somewhat restricted in content, and while they—as we hope—will present a fair summary of the present state of knowledge, not all papers covering the respective topics could be included.

Our interest in jaundice during pregnancy has been stimulated during our work as medical consultant and gastroenterologist to the Department of Obstetrics, Zurich University Hospital and I wish to gratefully acknowledge the continuing help and encouragement received from its chief Professor E. R. Held, his co-worker Dr. H. I. Wyss and from my chief Professor P. H. Rossier, head of the Department of Medicine, Zurich University Hospital.

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I The liver in normal pregnancy

The liver performs its function well during gestation. Tests and laboratory determinations usually employed to evaluate liver function and liver disease deviate however not infrequently from the normal in healthy pregnant women. These disturbances are rarely severe surpassing the accepted upper limit of normal for non pregnant females only slightly. They are on the main more common in the later weeks of gestation and are usually rectified after delivery.

The knowledge of the physiological derangements is important for any physician evaluating a women with jaundice during pregnancy. In the non pregnant state there exists for every test an accepted division line between a normal and a pathological result. It is probably wise to follow Friedberg's suggestion to form a third intermediary group of test results in pregnant women those lying above the upper limit of normal for non pregnant subjects but lying below the highest observed values in uncomplicated pregnancies. As only a certain percentage of pregnant females surpass the non pregnant norm this intermediary group may then be truly 'normal' or may have a pathological significance.

Reviews of liver function in uncomplicated pregnancy have been attempted by Holmer 1927, Vignes 1930, Dietel 1936, Williams 1932, Dieckmann 1932,

Lichtman 1933, Thorling 1935, Hoynck Van Papendrecht 1957, Richman 1960 and Friedberg 1960, 1962, 1963. A fair summing up has been made by Cross in 1929. 'The liver is the largest, the most abused, the most neglected, one of the most important, and the least understood organ of the body.'

Liver palpation

Palpation of the liver can be difficult in the later weeks of gestation, when the liver may be forced upwards, backwards and to the right by the enlarged uterus. A normal liver is rarely palpable towards term. When it is felt liver disease or congestive heart failure should be suspected.

Spider angiomas and palmar erythema

Bean et al carefully examined all women in a prenatal clinic during one year. They found spider angiomas in 66.6% of 48½ white and in 11.4% of 70.9 negro pregnant females. The control incidence was 12% among 38 non pregnant white females with children and 14.9% among 290 white soldiers. In the same pregnancy groups 62.5% of white and 35% of negro females had palmar erythema. There was an overlap in the occurrence of spider angiomas and palmar erythema in about two thirds of each group. Spider angiomas occurred as early as the second month of gestation.

Hemoglobin and serum iron

Young et al demonstrated a fall in hemoglobin levels during pregnancy in 219 serially examined patients. Hoch et al found in addition a fall in serum iron levels, but could not demonstrate a correlation between hemoglobin and serum iron. In a more detailed study of 176 patients Niesert observed an average fall in hemoglobin from 90% to 80%, a fluctuating but on the whole constant serum iron level, an increase in total iron binding capacity and a corresponding fall in iron saturation. Ilkonen found the serum iron levels widely dispersed (range 39—294 microgm per 100 ml) in his 84 normal pregnancies.

Anemia during the last trimester of pregnancy is generally considered to be present only when the hemoglobin level falls to below 10 gm per 100 ml. The fall in hemoglobin and hematocrit is explained by the rise in plasma volume which is only partially compensated by a minor rise in red cell volume (Tysoe and Lowenstein).

Total leucocyte and differential count

Kuvin and Brecher found a normal white cell count and morphology in only 46% of 111 pregnancies. 20% have an increase of total white cells above 10,000 per cu mm and counts of 15,000 per cu mm are still considered normal in the last trimester. Abnormal differential counts are also common. There is an increase in both segmented and nonsegmented neutrophils. Not infrequently myelocytes and metamyelocytes may be seen which are not necessarily part of the "shift" to the left.

Prothrombin time

The prothrombin time remains normal in all cases of uncomplicated pregnancy (Ilkonen).

Urinary bile components

Although most textbooks state that urinary urobilinogen and urobilin may be increased in the later stages of pregnancy, few exact studies have been performed. Merletti in 1902 observed as a rule a two to three fold increase of urobilin in the last trimester. In normal pregnancies at term Arfwedson found 'pathological bile components' in 5% of 100 patients, Dieckmann et al a positive urine bilirubin test in 14.5% of 85 patients and Labo et al a positive urine urobilin test in 8.3% of 75 patients. On the other hand Cross reported bilirubin, urobilin and urobilinogen tests to be all normal in 61 uncomplicated pregnancies.

Serum bilirubin

Total serum bilirubin levels were reported to be normal during uncomplicated pregnancy by Cross in 61 patients, by Cantarow et al in 34 patients, by Dieckmann et al in 83 patients, by Wetstone et al in 56 patients and by Thorling in 202 patients. The latter author gives a mean of 0.3 mg per 100 ml with a range of 0.1 to 1.1 mg per 100 ml.

Other authors report a mild serum bilirubin increase in a small percentage of normal pregnancies, e.g. Eufinger and Bader. Ilkonen found bilirubin levels of 1.0 to 2.0 mg per 100 ml in 2% of 100 patients, Arfwedson in 1% of 100 patients and McNair and Jaynes in 4.3% of 364 patients. Among Friedberg's 120

with a sharp incidence rise between the 2nd and 5th month and but a slow rise thereafter. During pregnancy the single spider angioma may increase in size and new ones may appear in the second and third trimester. Most disappear after delivery.

Bean et al's study is the result of a meticulous search for these skin changes. It is not surprising that they are usually not noted by the more hasty observer and that they are rarely mentioned in obstetric textbooks.

Histological changes in liver biopsies

In 1907 Hofbauer described as typical histological changes of the liver during pregnancy centrilobular fatty degeneration, decreased glycogen content, centrilobular bile stasis and ectasia of the centrilobular veins and capillaries. He coined the term "*Schwangerschaftsleber*". His opinion was based on 4 post-mortem examinations of pregnant women dying at term three from pulmonary emboli and one from exsanguination during delivery. This concept was challenged in 1910 by Schuckel but still kept appearing in the literature up to 1945. It is now clear that Hofbauer's observations were due to unrelated and terminal pathology in his 4 cases.

In 1945 Ingerslev and Teilum performed liver biopsies in 17 females during delivery. They found some variation in the size of liver cells and nuclei, occasionally small lymphocytic infiltrations in the portal spaces, and sometimes a mild vacuolar accumulation of fat in the centrilobular area which was more pronounced than in their 6 nonpregnant controls. Otherwise the liver histology

was considered normal. In 1947 Nixon et al biopsied 9 pregnant females and found only minor nonspecific histological changes. These consisted in an occasional variation in the shape of liver cells, in an increase in the number of large nuclei, in some irregularities of the nuclei, and in an increased glycogen content of the cytoplasm. In the same year Dietel obtained 31 surgical liver biopsies during pregnancy and found only a slight increase in the number of binucleated liver cells when compared with 50 non pregnant controls.

All these authors agree that histological liver changes during pregnancy are minor and nonspecific and that a "*Schwangerschaftsleber*" does not exist.

Liver blood flow

Liver blood flow is within the normal range in pregnancy. Using the brom-sulfalein technique and hepatic vein catheterization Munnell and Taylor found a mean liver blood flow of 1,534 ml per minute per 1.73 sq m of body surface in 13 pregnant females (range 1,075–2,465) compared to a mean of 1,348 ml per minute (range 1,177–1,900) in 15 nonpregnant controls.

This is noteworthy because in pregnancy plasma volume and blood volume rise by 50 to 60% and cardiac output increases by 30 to 50% reaching a maximum in the 7th pregnancy month and returning to normal towards term (Tysoe and Lowenstein). Liver blood flow comprises 35% of cardiac output in nonpregnant females and only 28% of cardiac output in pregnancy. The excess blood volume is shunted through the placenta.

TABLE 1 Mean serum alkaline phosphatase activity during uncomplicated pregnancy

Alk phosphatase method and units	Normal range	Month of gestation						Number of pat	References
		5	6	7	8	9	10		
King Armstrong	1-14	74	78	79	107	121	136	18	Mukherjee
Bodansky	1-4	—	29	32	36	47	59	300	Bodansky et al.
Buch & Buch	1-8	37	39	49	58	86	95	119	Thorling
Bessey Lowry	0.8-2.3	14	16	17	23	31	27	43	Beck and Clark
Roberts	0-6	30	33	41	83	104	125	201	Meranze et al
Vermehren	17-66	68	64	100	134	143	152	139	Vermehren
Shinowara Jones-Reinhart	2-9	32	34	39	47	114	118	239	Friedman et al

month differ markedly when compared to the respective upper limit of normal. The upper limit of normal is just reached with the King-Armstrong method. It is slightly surpassed in the Bodansky, Buch and Buch, Bessey-Lowry and the Shinowara-Jones-Reinhart technique and reaches twice the upper limit of normal with the Roberts and the Vermehren method. In individual women there may be little or no rise; in others the increase towards term is marked (Thorling, Beck and Clark). In the last two months 28% surpassed 14 King-Armstrong units (Mukherjee), 41% 20 King-Armstrong units (Young et al), 11% 5.5 Bessey-Lowry units (Ikonen), 60% surpassed 4 and 20% 8 Bodansky units (Bodansky et al).

Alkaline phosphatase rises again during labor, especially in the second stage. In 15 serially examined women Mukherjee found mean alkaline phosphatases of 13.5 King-Armstrong units during the tenth month, 13.7 units during the first stage of labor, 18.2 units during

the second stage of labor and 11.4 units on the 4th day after delivery. The underlying mechanism of this alkaline phosphatase rise is not clear. Generally it is felt to represent a physiological response to the demands made by the foetus during the last 3 months of gestation (Mukherjee). Beck and Clark believe that the excess phosphatase originates in the placenta. They found no increase of the alkaline phosphatase component which can be inhibited by sodium taurocholate and which is presumed to stem from bone and kidney. Alkaline phosphatase activity falls to pre-pregnancy levels within 4 to 6 weeks after delivery.

Serum transaminases and other serum enzymes

Serum glutamic oxalacetic transaminase remains normal throughout pregnancy (Mason and Wroblewski, extensive study, Borglin 34 cases, West and Zimmerman 70 cases, Knutson et al 100 cases, Crisp et al 30 cases, Stone et al 71 cases, Kubli 32 cases, Friedman et al

patients 15% had a serum bilirubin of 1.0 to 1.5 mg per 100 ml and 1.7% of more than 1.5 mg per 100 ml. Among Dieckmann et al's 85 patients with normal total bilirubin levels 20% had an increase of the 1 minute direct reacting fraction.

Neither Thorling nor McNair and Jaynes found a correlation between bilirubin levels and stage of gestation. There is no rise toward term, as does occur with alkaline phosphatase activity.

However, intravenous bilirubin tolerance tests are often impaired with advancing pregnancy. Among pregnant females with normal serum bilirubin levels Kaufmann found an increased bilirubin retention in 7 of 16 during the second half of pregnancy, Nurnberger in 13 of 25 toward the end of pregnancy, Soffer in 1 of 11 during the first trimester and in 9 of 10 during the second and third trimester, Sullivan et al in 1 of 11 during the first half and in 15 of 47 during the second half of pregnancy. Soffer examined 10 patients twice and found in 7 a definite increase in bilirubin retention during the latter part of gestation. No studies of this nature have been performed since 1934.

Bromsulfalein retention

Normal bromsulfalein excretion during pregnancy was found in two early reports in 67 (Siegal) and 61 patients (Cross) except during labor. A slightly increased bromsulfalein retention was not infrequently reported towards term in later series. Thus, Christliff and Bonsnes found a 5-10% retention at 45 minutes in 3 of 36 and Cantarow et al in 7 of 34 pregnancies at term. In

the third trimester Lobo et al observed a 7-12% retention in 7 of 75 pregnancies while Friedberg (1962) found a retention of more than 5% in 11% and of more than 10% in 3.7% of his patients. Merz bromsulfalein retention was reported by Wilken as 4% during months 4-6, as 9.7% during month 10 and as 16.3% during labor. He ascribed this increased retention during labor to a temporarily reduced liver blood flow.

Combes et al, using the prolonged bromsulfalein infusion technique of Wheeler, found in 15 women in the second half of pregnancy a mean increase in relative hepatic storage capacity (S) of 122% and a mean decrease of maximal excretory capacity (T_m) of 27% while the results in the same women in the first half of pregnancy were no different from 10 normal non-pregnant controls. After delivery, T_m is normalized before.

Galactose tolerance test

Urinary galactose excretion after an oral 40 g galactose load was found to be normal in 20 pregnancies at term by Nurnberger while others claim it to be 'often abnormal' (Friedberg 1960).

Serum alkaline phosphatase

Mean alkaline phosphatase activity rises slightly during the first half of pregnancy and then sharply during the seventh month, reaching a peak at term (Bodansky et al, Hoch et al, Speert et al). Mean values obtained by different methods are given in Table 1. With different techniques the trend is the same but mean activities during the tenth

In the detailed and serial observation of 21 women during their whole pregnancy by Coryell et al total serum proteins declined by 13 % towards term. The albumin/globulin ratio fell from 1.32 before pregnancy to 1.21 in the second trimester, to 0.84 in the third trimester and to a low of 0.7 a few days after delivery. On electrophoresis there was a fall in albumin and gamma globulin and a rise in the alpha 1, alpha 2 and beta globulin fractions as well as in fibrinogen. These changes disappeared 6 to 12 weeks following delivery.

The erythrocyte sedimentation rate increases from the sixth month until term to about 30 mm per hour, Winthrobe (Tysoe and Lowenstein). This is probably related to the increase in fibrinogen.

Serum turbidity and flocculation tests

The reports regarding incidence of positive turbidity or flocculation tests during pregnancy are conflicting.

The *thymol turbidity* was normal in all of Thorling's 201 patients and in the 27 patients of Christulf and Bonsnes. On the other hand Labo et al found 6.6 % abnormal reactions among 75 patients near term. Friedberg et al 10.3 % among 120 patients. Ilkonen 12 % among 100 patients. McNair and Jaynes 11 % in early and 15 % in late pregnancy among some 300 patients and Dieckmann et al 15 % positives in his 83 patients throughout pregnancy.

The *Takata reaction* was normal in all of Thorling's 197 cases and positive in 20 % of Friedberg's 120 patients. The latter also found 20 % positive *cadmium reactions*.

The *cephalin flocculation test* was normal in all of Thorling's 197 and Christulf and Bonsnes' 27 patients. Salmon found only 1 positive reaction in 71 patients. In contrast to these studies Labo et al observed 6.6 % positive tests among 75 patients, McNair and Jaynes 8 % among 300 at term and Day and Hellestrand even 22.5 % of 101 patients.

It is possible that the test results are highly influenced by the specific technique and test reagents used in the different laboratories. Dieckmann et al for instance reported in 1951 around 20 % positive *cephalin flocculation tests* among 85 women and even 40 % positives in the first trimester but 3 years later Dieckmann and Pottinger found only 5.2 % pathological reactions in 225 patients.

Conclusions

Of the many procedures used to evaluate 'liver function' only the serum transaminases and the prothrombin time remain within normal limits in all pregnant women. Liver blood flow remains quantitatively unchanged while cardiac output increases. Liver histology may show minor and nonspecific changes, but is generally considered to be within normal limits.

There is an increased incidence towards term in spider naevi and palmar erythema. The following laboratory test results are often increased progressively with advancing pregnancy: total white cell count, number of segmented and non segmented neutrophils, myelocytes and metamyelocytes, serum alkaline phosphatase, serum cholesterol, total serum lipids, bromsulfalein retention,

al 280 cases) Among Ikonen's 99 cases 96 were normal and 3 had SGOT levels between 40 and 80 units, all 3 being patients in labor. Mean SGOT levels during pregnancy are generally lower than in nonpregnant controls. Among West and Zimmerman's 70 cases none surpassed a value of 15 units during the first two trimesters. The means tend to rise toward term but remain within normal limits.

Serum glutamic pyruvic transaminase behaves identically (Rimbach and Bonow 25 cases, Ikonen 99 cases). Occasional elevations up to 80 units may be observed during labor.

Reports on *lactic dehydrogenase* during pregnancy are more conflicting. Normal values in all patients were reported by Linton and Miller (33 cases), Knutson et al (100 cases), and Kubli (44 cases). West and Zimmerman report an increase in 2 out of 70 cases, but Hill in 19 out of 40 and Stone et al in 9 out of 71 cases. During labor *lactic dehydrogenase* is elevated in nearly half the cases (West and Zimmerman 11 of 27, Kubli 13 of 33).

Serum ornithyl carbamyl transferase was slightly elevated in 7 of 16 normal pregnancies (Reichard et al).

Serum cholinesterase activity diminishes progressively throughout pregnancy (Friedman et al). Abnormally low values (less than 170 units) were found in 17% of patients during the first trimester, in 33% during the second trimester and in 43% during the third trimester (Wetstone et al).

Serum tributyrinase also shows a slight decrease in mean activity towards term (Friedman et al).

Serum cholesterol and serum lipids

Serum cholesterol levels begin to rise in about the 4th pregnancy month and usually reach a maximum in the 8th month. McNair and Jaynes found values over 250 mg per 100 ml in 9% of normal pregnancies in the 4th month, in 53% in the 6th month and in 57% in the 8th month. Similar findings were obtained by Wetstone et al. In the third trimester 6 of their 11 patients had serum cholesterol levels over 300 mg per 100 ml with a maximum of 435 mg per 100 ml. Serum cholesterol varied from 250 to 510 mg per 100 ml in 100 females with uncomplicated pregnancy near term reported by Ikonen, with the majority in the range of 300 to 420 mg per 100 ml. Von Studnitz, in a careful study of 101 females during the course of their pregnancy, found also an increase in total lipids, alpha lipoproteins, beta lipoproteins and phospholipids. These changes are presumed to be due to hormonal influence.

Total serum proteins and serum electrophoresis

Total serum protein levels fall gradually during pregnancy, reaching a low at term (Pfau, Wetstone et al, McNair and Jaynes). In a series of over 300 pregnant women McNair and Jaynes found serum protein levels below 6 gtu per 100 ml in 7% of patients during the 2nd pregnancy month and in 43% during the 8th month. It must be remembered that during pregnancy circulating plasma volume increases by about 50-60% and total body water by about 20%.

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hepatic bromsulfalein storage, serum alpha and beta globulins, fibrinogen and erythrocyte sedimentation rate

An occasional increase not dependant on the stage of gestation is seen in serum bilirubin (up to 2 mg per 100 ml) and in the incidence of pathological serum turbidity and flocculation reactions. No careful studies exist on the incidence of pathological bile components in the urine. It is suggested that occasionally bilirubin, urobilin or increased amounts of urobilinogen may be present in uncomplicated pregnancy.

There is a decrease towards term in hemoglobin, erythrocyte count, hemato-

crit, total serum proteins, serum albumin, gamma globulin, serum cholinesterase, intravenous bilirubin tolerance and maximal bromsulfalein excretory capacity (T_m).

With the exception of total white counts most deviations from the normal are minor. The main diagnostic difficulties will be encountered with elevated alkaline phosphatase levels and pathological turbidity and flocculation tests. Determination of serum bilirubin is the single most valuable test during pregnancy, although a normal value does not exclude the presence of liver disease.

II Jaundice during pregnancy

1) Incidence of jaundice during pregnancy

Jaundice during pregnancy is rare. Table 2 is an attempt to summarize all reports in the literature from which incidence can be calculated. The overall figure is 537 cases of jaundice in 822 842 pregnancies an incidence of 0.067% or cases per 10 000 pregnancies or 1 case per 1500 pregnancies. The total series has been subdivided in 2 parts: 10 reports which give hepatitis as only diagnosis and 11 reports which list different etiologies including every case of jaundice during pregnancy. As can be seen incidence of jaundice is slightly higher in the series which list hepatitis only. This does not make much sense. It may indicate that total incidence of jaundice during pregnancy reflects mainly the frequency of hepatitis during pregnancy in the respective series or that reports are mainly written after the experience of a hepatitis epidemic. Hepatitis accounts for at least 41% of all cases of jaundice during pregnancy (see Table 4). This figure is in some areas during certain time periods approaching 100.

In the single reports incidence of jaundice during pregnancy varies between 2 per 10 000 and 3 per 1 000 pregnancies with the exception of 3 series collected during the height of a hepatitis epidemic. The highest observed

incidence in reports covering more than a one year period is 8 hepatitis cases per 1 000 pregnancies (Zondek and Bromberg).

Excluding again series with observation periods of one year or less the incidence of jaundice per year varies between 0.3 and 10.6 cases with an average of 3 cases per year (415 cases in 138 years).

2) Classification of jaundice during pregnancy

The oldest classification of jaundice during pregnancy into *icterus levis* (those who survive) and *icterus gravis* (those who die) originates probably from French (1838) and is still used in the French literature with the addition of a third category: *ictère pseudo-grave* (those who look like they are going to die but eventually survive). Such a grouping is certainly clear cut but helpful to the clinician only in retrospect.

More meaningful is an attempt at an etiologic classification separating jaundice caused by pregnancy per se (only seen in pregnant women) from jaundice occurring by chance during the course of an otherwise uncomplicated gestation (seen also in non pregnant females and in males). The classification given in

hepatic bromsulfalein storage, serum alpha and beta globulins, fibrinogen and erythrocyte sedimentation rate

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II Jaundice during pregnancy

1) Incidence of jaundice during pregnancy

Jaundice during pregnancy is rare. Table 2 is an attempt to summarize all reports in the literature from which incidence can be calculated. The overall figure is 557 cases of jaundice in 822,842 pregnancies, an incidence of 0.067 ‰ or 7 cases per 10 000 pregnancies or 1 case per 1 500 pregnancies. The total series has been subdivided in 2 parts: 10 reports which give hepatitis as only diagnosis and 11 reports which list different etiologies including every case of jaundice during pregnancy. As can be seen, incidence of jaundice is slightly higher in the series which list hepatitis only. This does not make much sense. It may indicate that total incidence of jaundice during pregnancy reflects mainly the frequency of hepatitis during pregnancy in the respective series or that reports are mainly written after the experience of a hepatitis epidemic. Hepatitis accounts for at least 41 ‰ of all cases with jaundice during pregnancy (see Table 4). This figure may in some areas during certain time periods approach 100 ‰.

In the single reports incidence of jaundice during pregnancy varies between 2 per 10 000 and 3 per 1 000 pregnancies, with the exception of 3 series collected during the height of a hepatitis epidemic. The highest observed

incidence in reports covering more than a one year period is 8 hepatitis cases per 1 000 pregnancies (Zondek and Bromberg).

Excluding again series with observation periods of one year or less, the incidence of jaundice per year varies between 0.3 and 10.6 cases, with an average of 3 cases per year (415 cases in 138 years).

2) Classification of jaundice during pregnancy

The oldest classification of jaundice during pregnancy into *icterus levis* (those who survive) and *icterus gravis* (those who die) originates probably from French (1838) and is still used in the French literature with the addition of a third category: *ictère pseudo-grave* (those who look like they are going to die but eventually survive). Such a grouping is certainly clear-cut but helpful to the clinician only in retrospect.

More meaningful is an attempt at an etiologic classification separating jaundice caused by pregnancy per se (only seen in pregnant women) from jaundice occurring by chance during the course of an otherwise uncomplicated gestation (seen also in non pregnant females and in males). The classification given in

TABLE 2 Incidence of jaundice during pregnancy

Year	Authors	Country	Time period (years)	Total preg nancies	Patients with jaundice	Incidence of jaundice		
						in % of all pregn	cases per year	
Series with hepatitis as only diagnosis								
1943	Saurer	Zurich	1	2 764	3	0 108	—	
1947	Zondek & Bromberg	Jerusalem	9	12 360	3	0 024	0 3	
	Zondek & Bromberg	Jerusalem	2 6	3 382	27*	0 798	10 1	
1950	Dill	Washington D C	3	25 000	12	0 048	4 0	
1951	Mickal	New Orleans	9	69 186	15	0 022	1 7	
1953	Martini et al	Hamburg	3 5	91 735	37	0 040	10 6	
1955	Paul	Toronto	15	46 000	10	0 022	0 7	
1956	Phatak & Patel	India	0 3	959	29	3 024	—	
	Phatak & Patel	India	0 3	1 370	6	0 438	—	
1957	Lacomme	Paris	2	10 000	10	0 100	—	
1959	Mazaud et al	Dakar	3	3 969	10	0 252	3 3	
1959	Peretz et al	Haifa	8	21 000	65	0 310	8 1	
	Combined		53+	287 725	227	0 078	3 4	
Series with jaundice of different etiology								
1951	Javert & Morrison	New York	2	74 087	51	0 069	—	
1955	Thorling	Uppsala	10	25 797	72	0 279	7 2	
1955	Meyer	Halle	1	1 540	18	1 161	—	
1957	Vincent	New Orleans	17	136 179	32	0 023	1 9	
1957	Enrile et al	Philippines	7	14 944	7**	0 047	1 0	
1961	Samuels	New Orleans	10	20 000	8	0 040	0 8	
1962	Cahill	New York	28	110 378	52	0 047	1 8	
1962	Synodinos et al	Athens	6	44 000	37	0 084	6 2	
1962	Cremone	Torino	2	9 870	6	0 061	3 0	
1963	Siegler & Keyser	New York	(7 10)	80 356	25	0 031	—	
1966	Haemmerli & Wyss	Zurich	5	17 956	22	0 122	4 4	
	Combined		85+	535 117	330	0 061	2 8	
Both collective series combined				138+	822 842	557	0 067	3 0

* 20 cases in report 2 with anicteric hepatitis have been excluded

** 8 cases in report 1 case of acute cholecystitis without jaundice is excluded

+ Excluding series with collection periods of 1 year or less

TABLE 3 Jaundice during pregnancy
(Synonym *icterus gravidarum*)

A. Jaundice in pregnancy

(Synonyms *icterus in graviditate* *concomitant jaundice*, *coincidental jaundice*, *ictere intercurrent*)

I Usual forms of jaundice occurring also in non pregnant subjects

- 1 Hepatic parenchymal disease (especially viral hepatitis)
- 2 Intrahepatic cholestasis (i.e. drug jaundice)
- 3 Extrahepatic cholestasis (i.e. common duct stones)
- 4 Congenital idiopathic hyperbilirubinaemias
- 5 Hemolytic disorders

II Jaundice in typical medical complications of pregnancy

- 1 Jaundice in severe pyelonephritis
- 2 Jaundice in pyelonephritis and tetracycline toxicity
- 3 Delayed chloroform poisoning
- 4 Jaundice after (criminal) abortions
(*Clostridium perfringens* septicemia, quinine toxicity etc.)

B Jaundice of pregnancy

(Synonyms *icterus e graviditate* *icterus graviditatis*, *icterus peculiar to pregnancy* *ictere lie à la grossesse*)

I Idiopathic jaundice of pregnancy

- 1 Intrahepatic cholestasis of pregnancy
(jaundice of late pregnancy recurrent jaundice of pregnancy)
- 2 Acute fatty metamorphosis of pregnancy
(obstetric acute yellow atrophy)

II Jaundice as a complication of another disease linked to pregnancy

- 1 Jaundice in hyperemesis gravidarum
- 2 Jaundice in vomiting of late pregnancy
- 3 Jaundice in severe toxemia of pregnancy
- 4 Jaundice in megaloblastic anemia of pregnancy
- 5 Jaundice in hemolytic anemia of pregnancy

Table 3 seems to represent best our present state of knowledge

Reviews of jaundice during pregnancy have been published by Mayer 1906 Kehrler 1907 Schuckele 1910 Seitz 1916 Rissmann 1917 Eppinger 1923 Holmer 1927 Chabrol 1932 Eppinger 1937 Verhage 1940 Seitz 1948 Puyo 1953 Lichtman 1953 Dietel 1954 Caroli et al 1954 Florling 1955 Lacomme 1957 Birt and Senzre 1957 and 1958 Wilken

1958 Cattan and Cattan 1959 Dominici 1960 Richman 1960 Friedberg 1960 Labby 1960 Sheehan 1961, Meeroff 1961 Boquen et al 1961, Cremona and Voghera 1962 Cahill 1962 Synodinos et al 1962 Synodinos 1963, Friedberg 1963 Sherlock 1963, Ikonen 1964 and Gerl and Bonow 1964 The following reviews fail to mention recurrent jaundice of pregnancy (intrahepatic cholestasis of pregnancy) Winter 1890

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1955	Paul	Toronto	15	46 000	10	0 022	0 7	
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	Phatak & Paul	India	0 3	1,370	6	0 438	—	
1957	Lacomme	Paris	?	10 000	10	0 100	—	
1959	Mazaud et al	Dakar	3	3 969	10	0 252	3 3	
1959	Peretz et al	Haifa	8	21 000	65	0 310	8 1	
	Combined		53+	287,725	227	0 078	3 4	
Series with jaundice of different etiology								
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1955	Thorling	Uppsala	10	25 797	72	0 279	7 2	
1955	Meyer	Halle	1	1 550	18	1 161	—	
1957	Vincent	New Orleans	17	136,179	32	0 023	1 9	
1957	Enrile et al	Philippines	7	14 944	7**	0 047	1 0	
1961	Samuels	New Orleans	10	20 000	8	0 040	0 8	
1962	Cahill	New York	28	110 378	52	0 047	1 8	
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	Combined		85+	535 117	330	0 061	2 8	
Both collective series combined				138+	822 842	557	0 067	3 0
					415+			

* 29 cases in report 2 with anicteric hepatitis have been excluded

** 11 cases in report 1 case of acute cholecystitis without jaundice is excluded

+ Excluding series with collection periods of 1 year or less.

TABLE 5 Jaundice during pregnancy Frequency distribution of different diseases in 15 series combined (456 cases)

Diagnosis	Number of cases	%
Jaundice in pregnancy		
1 Hepatocellular jaundice		
Infectious hepatitis	189	212
Acute yellow atrophy non-specified	7	
Erythraemia	4	
Leptospirosis (Weils disease)*	1	
Liver metastasis*	1	
Tuberculosis of liver*	1	
Echinococcus of liver*	2	
Chlorpromazine jaundice***	6	
P.A.S. jaundice	1	
2 Extrahepatic obstruction		
Common duct stones	27	28
Adenocarcinoma of common duct*	1	
3 Familial non hemolytic jaundice		2
4 Hemolytic jaundice		
Sickle cell anemia	8	19
After blood transfusion	4	
Hemoglobin disease	1	
Familial hemolysis (Spherocytosis)**	2	
Not specified	4	
5 Mixed forms		
Leukemia	2	6
Appendicitis with perforation*	1	
Infected cyst adenoma of ovary	1	
Septic shock	2	
Jaundice in complications of pregnancy		
Eclampsic gra. durum	27	60
Eclampsia	21	
Marked hyperemesis without eclampsia	3	
Severe preeclampsia with jaundice	5	
After stillborn or abortion	1	
After normal abortion	3	
Jaundice of pregnancy		
Intrahepatic cholestasis of pregnancy	65	90=
Recurrent intrahepatic cholestasis of pregnancy	29	
Acute fatty metamorphosis of pregnancy	2	
Undiagnosed		33=
		456=
		100.0

References for table 5

(a) 1. Greig & Barry & D. Haver

Jaundice & Morrison

Sanchez

Non et al.

* Enrie et al.

Segler & Acjner

References for unnumbered diagnoses are given in table 4

Vincent

Verhage

Thorling

* Synodinos et al.

Thorling

TABLE 4 Jaundice during pregnancy Frequency distribution of different diseases in single reports

Year	Author	Total cases with jaundice	Hepatitis	Common duct stones	Hemolysis	Hyperemesis	Eclampsia	Cholestasis of pregnancy	Recurrent cholestasis of pregnancy	Other*	No diagnosis
1940	Verhage	43	6	2	1	15	14	—	1	4	—
1947	Nixon et al	12	9	—	—	—	1	—	—	1	1
1951	Javert & Morrison	51	20	9	—	3	—	—	—	13	—
1955	Thorling	72	24	—	—	6	—	28	3	7	4
1955	Barry & O Dwyer	9	5	—	—	—	1	1	—	2	—
1955	Meyer	18	8	1	1	3	3	—	2	—	—
1957	Vincent	32	25	2	—	—	—	—	—	5	—
1957	Ennle et al	7	3	1	—	—	1	—	—	2	—
1961	Samuels	8	6	1	—	—	—	—	—	1	—
1962	Cahill	52	29	3	—	—	1	—	4	1	14
1962	Synodinos et al.	37	27	—	9	—	—	—	—	1	—
1962	Cremona	6	4	—	—	—	—	—	—	2	—
1963	Siegler & Keyser	25	12	2	1	—	—	1	—	3	4
1964	Ikonen	62	5	5	1	—	—	33	13	2	1
1966	Haemmerli & Wyss	22	6	1	—	—	—	—	6	—	9
	Combined series	456	189	27	19	27	21	63	29	46	33
	in %	100	41.5	5.9	4.2	5.9	4.6	14.2	6.4	10.1	7.2

* Detailed in table 5

von Winckel 1893, Vinay 1894, Quirno et al 1948, Lock et al 1953, Puder 1955, Williams 1957, Millen 1957, Imparato 1958 and Varangot 1962. By far the most outstanding attempt at a clinical differential diagnosis is provided in Thorling's monography (1955).

3) Frequency distribution of different diseases causing jaundice during pregnancy

Fifteen reports including 11 used in Table 2, break down their cases of jaundice during pregnancy into different

etiologic categories. The 456 cases are summarized in tables 4 and 5. It is clearly evident that infectious hepatitis constitutes the most frequent single disease entity. The figure of 41.5 % would be much higher, if the reports listing hepatitis as only diagnosis had been included. Many of these hepatitis reports exclude just a few cases of other etiologies while some authors seem to feel that every case of jaundice during pregnancy is caused by viral hepatitis.

Table 4 lists the etiologic distribution reported by the individual authors.

TABLE 6 Incidence of viral hepatitis in relation to stage of gestation

Year	Authors	Cases of hepatitis			
		Total	1 Trm	2 Trm	3 Trm
1951	Ingerslev & Teisum	88	2	1	85
1951	Javert & Morrison	20	5	2	13
1951	Nickal	15	0	4	11
1954	Ellegast et al.	52	14	21	17
1954	Frucht & Metcalfe	17	0	2	15
1955	Thorling	24	5	12	7
1955	Long Boysen & Priest	10	1	3	6
1955	Hartmann & Schoen	26	6	10	10
1956	Ezer & Bourdon	34	2	25	7
1957	Dorfler	55	10	25	20
1959	Peretz et al.	65	7	25	33
1961	Denning & Bruckmann	21	7	4	10
1967	Cahill	29	8	6	15
	Total	456	67	140	249
	in %	100	14.7	30.7	54.6

4) Review of literature on jaundice during pregnancy

The following chapters are an attempt to summarize our present state of knowledge concerning the different forms of jaundice during pregnancy by a review of the available world literature

Infectious hepatitis during pregnancy

The older literature and even some of the newer textbooks contain the following statements pregnant women are especially susceptible to hepatitis hepatitis in pregnant women occurs most frequently in the last trimester of pregnancy hepatitis runs a severe course during pregnancy and results in a very high mortality All these statements do not bear out under a critical evaluation of the literature

Susceptibility of pregnant women to viral hepatitis The incidence of hepatitis in pregnant women runs parallel to epidemics in the general population This is shown clearly in 5 reports Zondek and Bromberg in Jerusalem observed from 1934 to 1943 only 3 cases in 12 360 pregnancies, while in a 32 months period during 1943—1946 they saw 29 cases among 3,382 pregnancies Peretz et al reported 65 cases of hepatitis in Haifa during 1950 to 1957 33 of which occurred during 1950/1951 Ingerslev and Teisum in Copenhagen observed 15 cases during 1928 to 1940 and 91 cases during 1941 to 1949 Phatak and Patil in India reported in two successive 4 months periods 29 cases among

These figures are in all likelihood not accurate. They may reflect the trends in diagnosis by different clinicians and in different countries rather than actual incidence. For example, 63 out of the 65 cases of non recurrent intrahepatic cholestasis of pregnancy are reported by two authors (Thorling and Ikonen). One case in our table is listed as 'hepatitis' in the original report, but has been otherwise classified by us on the basis of the detailed published data (case 1 in Barry and O'Dwyer). It is very likely that many cases of intrahepatic cholestasis of pregnancy are hidden under the diagnosis "hepatitis", especially in series where this is the only diagnosis made. For instance 3 of 6 biopsies in Ingerslevs and Teilmann's series of 91 "hepatitis" and 1 of 6 biopsies in the 65 'hepatitis' of Peretz et al showed 'normal liver tissue, which is frequently seen in intrahepatic cholestasis of pregnancy. Intrahepatic cholestasis of pregnancy may possibly also be reported under jaundice due to choledocholithiasis, because the laboratory findings in both diseases may be identical. It is interesting in this regard, that 9 out of 27 cases with jaundice ascribed to common duct stones are contained in one single report. A rather interesting observation is that only 6 of the 15 reports admit to having cases of jaundice where no definite diagnosis could be reached.

Trends inherent in the time period during which the report was written may also influence the etiologic frequency distribution. Of course no case of intrahepatic cholestasis of pregnancy will be reported before 1955, the year of Thorling's description of the disease. Cases of

jaundice due to choledocholithiasis are few in single series after 1951, perhaps due to the more wide spread use of cholangiography. More than half of the cases of jaundice second to hyperemesis or eclampsia are reported by a single author in 1940 (Verhage). It is possible that these disorders are better treated today and do less frequently progress to a jaundiced stage.

Geographic pathology also influences frequency distribution. Nine of 19 cases due to hemolytic jaundice are reported from Athens, due to the frequency of sickle cell anemia among the population admitted to that particular hospital. The same holds true for jaundice due to echinococcus of the liver.

Table 5 lists all diagnosis made in the 15 reports. It may serve as a survey of the rarer types of jaundice occasionally encountered. It is interesting to note that among the 19 cases with hemolytic jaundice there is a conspicuous absence of "idiopathic hemolytic anemia of pregnancy" and of 'pernicious anemia of pregnancy', two diseases quoted in most textbooks.

Few valid conclusions may be drawn from this type of survey. The diagnosis of hepatitis is probably made too often and on inconclusive evidence but still viral hepatitis will account for the majority of cases with jaundice during pregnancy. Rarer than generally thought are obstructive jaundice due to common duct stones and hemolytic jaundice due to the state of gestation. Wrong diagnosis are probably made most often in cases of intrahepatic cholestasis of pregnancy unless when recurrent in successive pregnancies.

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1954	Fruht & McCallie	17	0	2	15
1955	Thong	24	5	12	7
1955	Long Boysen & Liles	10	1	3	6
1955	Hannemann & Schoen	26	6	10	10
1956	Ezer & Bouillon	34	2	25	7
1957	Durfler	55	10	25	20
1959	Perez et al	65	7	25	33
1961	Denning & Bruckmann	21	7	4	10
1962	Cabell	29	8	6	15
	Total	456	67	140	249
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The following chapters are an attempt to summarize our present state of knowledge concerning the different forms of jaundice during pregnancy by a review of the available world literature.

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The older literature and even some of the newer textbooks contain the following statements: pregnant women are especially susceptible to hepatitis; hepatitis in pregnant women occurs most frequently in the latter trimester of pregnancy; hepatitis runs a severe course during pregnancy and results in a very high mortality. All these statements do not bear out under a critical evaluation of the literature.

Susceptibility of pregnant women to viral hepatitis The incidence of hepatitis in pregnant women runs parallel to epidemics in the general population. This is shown clearly in 3 reports: Zondek and Bromberg in Jerusalem observed from 1934 to 1943 only 3 cases in 17360 pregnancies, while in a 32 months period during 1943-1946 they saw 79 cases among 3382 pregnancies. Perez et al reported 65 cases of hepatitis in Haifa during 1950 to 1957, 33 of which occurred during 1950/1951. Ingerslev and Telum in Copenhagen observed 13 cases during 1928 to 1940 and 91 cases during 1941 to 1949. Phatak and Patil in India reported in two successive 4 months periods 79 cases among

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TABLE 8 Clinical course in viral hepatitis during pregnancy

Author	Total cases	Clinical course of hepatitis			
		Mild	Moderate	Severe	Deaths
Schubert & Peters	26	17		9	1
Durfler	60	29	29	II	1
Ellegast et al.	69	46	17	6	1

debility may play the same role as in the series from the Mediterranean area and alcoholism could be an additional factor

Clinical course of hepatitis during pregnancy Statements regarding the clinical course of hepatitis during pregnancy by different authors vary according to the reported mortality in their series. Mortality as we have already pointed out depends mostly on the general condition of the patient maternal and not on the disease itself. The same geographic subdivision as for the mortality statistics seems indicated when discussing clinical course.

The severe cases from the Mediterranean area and Asia are not quite uniform. In the series of Ezis and Bourdon all 22 lethal cases were admitted in coma and died very rapidly. 7 of them even before delivery. Eleven delivered stillborn children and 4 premature living babies, 3 of which died shortly thereafter. Bilirubin levels in the mothers varied between 2.9 and 10 mg per 100 ml, alkaline phosphatases between 11 and 17 Bodansky units and blood urea nitrogen between 16 and 31 mg per 100 ml. Outstanding features were very high white blood cell counts (25 to 30,000)

low blood sugars (between 14 and 40 mg per 100 ml in most) and very low prothrombin times (5 to 23 %) (Houel et al., same cases reported by different authors 3 years later). There was usually diffuse and massive bleeding from the gastrointestinal tract without obvious source at postmortem examination.

In the other series some of the comatous patients survived: 1 of 8 with coma reported by Corcos, 7 of 14 reported by Pinnoli, 5 of 16 reported by Phatak and Paul and 4 of 9 reported by Zondek and Bromberg. The latter authors stress as ominous signs the onset of tachycardia and a sudden drop in blood urea nitrogen associated with a rise in serum amino acids and a slight rise in non-protein nitrogen (to 38—40 mg per 100 ml). In the series from India serum bilirubin levels were strikingly low (2.0 to 6.6 mg per 100 ml).

Liver coma usually precipitates a spontaneous abortion. A spontaneous abortion in a severe case of hepatitis with or without coma does however, not ameliorate the disease. In fact 3 of Corcos' patients developed lethal coma only a week after their abortion. Most authors agree today that hepatitis in pregnancy is no indication for a therapeutic abortion.

from obstetrical departments which are for obvious reasons largely composed of patients in the later stage of pregnancy. The series of Ingerslev and Teilum illustrates this best. Patients with hepatitis in the first trimester of pregnancy are more likely to be referred to other departments, as at this stage pregnancy itself does not yet take predominance in the handling of the patient. In most likelihood hepatitis occurs with equal incidence in all trimesters of pregnancy, as is demonstrated in the two large series collected in departments of internal medicine (Ellegast et al, Dorflier)

Mortality from hepatitis during pregnancy. Table 7 gives the mortality figures for viral hepatitis during pregnancy collected from 38 reports in the literature. The overall figure of 92 deaths among 887 cases or 10.4% appears markedly higher than the mortality in the general population from the same disease. A more meaningful interpretation of the data is achieved by dividing the reports geographically.

In Europe, a mortality of 18% among 449 cases is certainly comparable to the usual mortality figures from hepatitis in the general population. Only one series shows a high mortality with 25% or 2 out of 8 patients (Dietel). Here a selection of cases must play an important role, because Martini et al report from the same town and from the same time period a series with 57 cases and a mortality of only 1.7%.

In marked contrast to the low mortality in Europe is the high mortality in the Mediterranean area (24.1%) and in Asia (50%). The mortality is high

in all series except the one from Istanbul. This cannot be due to chance. An especially virulent virus in that area could be a possible explanation. Peretz et al report that in Israel the new immigrants are not immunized to the middle East strain of hepatitis virus. More likely, the high mortality reflects the general debility and undernourishment of the indigene population. The 22 deaths from the series from Algiers occurred all in moslem women, who were brought to the hospital already in coma and died within hours to 4 days. In the report by Mazaud et al from Dakar 3 of 6 African women died, but none of 4 European females. Zondek and Bromberg state that among their 27 patients 18 were definitely undernourished, including the 9 cases with a severe course. Their 5 deaths occurred in a 5 months period during the height of a virulent epidemic.

French authors often state that the combination of pregnancy and hepatitis has a very grave outlook. This view is readily understandable on the basis of the 3 reported French series. 29 of 34 patients died, a mortality of 53.7%. However, these 3 series originate from Dakar, Tunis and Algiers, and no report on this topic has ever been published from the French mainland.

The series from the United States show an overall mortality of 7.7%, but the single reports have either a very low or a very high mortality. This reflects probably the different hospital types in that country, admitting different population groups. In hospitals serving mainly people of the lowest income groups, undernourishment and general

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In the other series some of the comatous patients survived: 4 of 8 with coma reported by Corcos, 7 of 14 reported by Pinnoli, 5 of 16 reported by Phatak and Paul and 4 of 9 reported by Zondek and Bromberg. The latter authors stress as ominous signs the onset of tachycardia and a sudden drop in blood urea nitrogen associated with a rise in serum amino acids and a slight rise in non-protein nitrogen (to 38–40 mg per 100 ml). In the series from India serum bilirubin levels were strikingly low (2.0 to 6.6 mg per 100 ml).

Liver coma usually precipitates a spontaneous abortion. A spontaneous abortion in a severe case of hepatitis with or without coma does however not ameliorate the disease. In fact 3 of Corcos' patients developed lethal coma only a week after their abortion. Most authors agree today that hepatitis in pregnancy is no indication for a therapeutic abortion.

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Mortality from hepatitis during pregnancy. Table 7 gives the mortality figures for viral hepatitis during pregnancy collected from 38 reports in the literature. The overall figure of 92 deaths among 887 cases or 10.4% appears markedly higher than the mortality in the general population from the same disease. A more meaningful interpretation of the data is achieved by dividing the reports geographically.

In Europe, a mortality of 18% among 449 cases is certainly comparable to the usual mortality figures from hepatitis in the general population. Only one series shows a high mortality with 25% or 2 out of 8 patients (Dietel). Here a selection of cases must play an important role, because Martini et al. report from the same town and from the same time period a series with 57 cases and a mortality of only 1.7%.

In marked contrast to the low mortality in Europe is the high mortality in the Mediterranean area (24.1%) and in Asia (50%). The mortality is high

in all series except the one from Istanbul. This cannot be due to chance. An especially virulent virus in that area could be a possible explanation. Peretz et al. report that in Israel the new immigrants are not immunized to the middle East strain of hepatitis virus. More likely, the high mortality reflects the general debility and undernourishment of the indigene population. The 22 deaths from the series from Algiers occurred all in Moslem women, who were brought to the hospital already in coma and died within hours to 4 days. In the report by Mazaud et al. from Dakar 3 of 6 African women died, but none of 4 European females. Zondek and Bromberg state that among their 27 patients 18 were definitely undernourished, including the 9 cases with a severe course. Their 5 deaths occurred in a 5 months period during the height of a virulent epidemic.

French authors often state that the combination of pregnancy and hepatitis has a very grave outlook. This view is readily understandable on the basis of the 3 reported French series: 29 of 54 patients died, a mortality of 53.7%. However, these 3 series originate from Dakar, Tunis and Algiers, and no report on this topic has ever been published from the French mainland.

The series from the United States show an overall mortality of 7.7% but the single reports have either a very low or a very high mortality. This reflects probably the different hospital types in that country admitting different population groups. In hospitals serving mainly people of the lowest income groups, undernourishment and general

TABLE 8 Clinical course in viral hepatitis during pregnancy

Author	Total cases	Clinical course of hepatitis			
		Mild	Moderate	Severe	Deaths
Schubert & Peters	26	17		9	1
Dorfler	60	29	29	2	1
Ellengast et al.	69	46	17	6	1

debility may play the same role as in the series from the Mediterranean area, and alcoholism could be an additional factor

Clinical course of hepatitis during pregnancy Statements regarding the clinical course of hepatitis during pregnancy by different authors vary according to the reported mortality in their series. Mortality as we have already pointed out depends mostly on the general condition of the patient maternal and not on the disease itself. The same geographic subdivision as for the mortality statistics seems indicated when discussing clinical course.

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In the reports from Europe, hepatitis during pregnancy is said to run a course no different from that in nonpregnant females or in males. In Martini et al.'s series bilirubin levels averaged 6.7 mg per 100 ml (range 0.7 to 24 mg per 100 ml). The only death occurred in a 38 year old woman with severe pyelonephritis. Thorling's 24 cases were all benign. Mean duration of hospitalisation was 24 days. Three authors classify their cases according to the severity of the clinical course (see Table 8).

Fllegast et al. compare the course in their 69 pregnant females with 360 nonpregnant hospitalised hepatitis cases. Mild forms were seen in 66.7 % of pregnant and 14.4 % of nonpregnant subjects, severe cases in 8.7 % of pregnant and in 15.8 % of nonpregnant patients. The 25 cases reported by Hartmann and Schoen were especially mild, with mean serum bilirubin levels of only 1.7 mg per 100 ml and an average hospitalisation of 19 days. Ingerslev and Teilum note also a high proportion of mild forms among their 91 patients. They further state that in 69 patients jaundiced up to the time of delivery the postpartum course was surprisingly uniform, in as much as jaundice cleared pretty regularly on the 11th post partum day regardless of the duration of jaundice before delivery. This last feature is unusual for hepatitis during pregnancy and it is likely, that this series from Copenhagen includes many cases of intrahepatic cholestasis of pregnancy which was still unknown in 1951. This assumption is supported by the fact that of their 6 liver biopsies only 3 showed hepatitis and the other 3 were normal.

Furthermore, the thymol turbidity test was normal in the majority of cases.

It is possible that other series may be heavily weighed in favour of mild forms of hepatitis during pregnancy by undiagnosed cases of intrahepatic cholestasis of pregnancy. Still the conclusion appears valid, that the course of hepatitis — at least in Europe — is not influenced by pregnancy.

Pregnancy itself is influenced by hepatitis only by a tendency to premature delivery. Despite the often low prothrombin time in hepatitis uterine bleeding during delivery is very rarely marked, not even in patients in hepatic coma (Zondek and Bromberg). Ingerslev and Teilum note that 16 % of their 91 patients lost more than 500 ml blood during delivery, while 11 % appears to be the norm in their hospital. However, no case bled excessively.

Sequellae from hepatitis during pregnancy. Follow up studies in women with hepatitis during pregnancy have been performed in 10 cases by Michal, in 26 by Hartmann and Schoen and in 57 by Martini et al. No evidence of liver pathology was detected. Five of Martini et al.'s patients had subjective liver symptoms but were found to have completely normal liver functions.

The only disagreeing report is that of Frucht and Metcalfe, who re-examined 11 women and found only 2 free of liver disease. One can hardly concur with this conclusion on the basis of their data. Five women had 'enlarged livers', five had bilirubin levels of 1.0 to 1.8 mg per 100 ml and 4 had slightly increased globulin levels (in two of these deter-

TABLE 9 Fate of infants born from mothers with viral hepatitis

Author	Total cases of hepatitis	Spontaneous abortions	Premature deliveries	Deaths of premature babies	Deaths in deliveries at term
Dietel	11	0	1	0	
Martini	54	2	11	0	
Ellegast et al.	47	0	16	4	2
Hartmann & Schoen	26	1	5	1	
Thorling	22	1	0	0	
Dorfler	55	3	10	1	
Denning & Bruckmann	19	1	2	0	
Total Europe	229	8	45	6	2
Zondek & Bromberg	29	0	7	8	
Pinnoli	37	1	16	0	
Synodinos et al.	20	0	1	1	1
Total Mediterranean	86	1	24	10	1
Mickal	13	0	1	1	
Roth	16	2	0	0	1
Paul	10	0	5	3	
Cahill	29	2	3	1	
Total North America	68	4	9	5	1
Total combined series	383	13	78	21	4
in	100	3.4	20.4	5.5	1.0

mined during a new pregnancy) However bromsulphalein excretion and flocculation tests were normal in all Similarly Ley and Liebl examined 42 women 2 to 11 years after their pregnancy with hepatitis They found minor chemical abnormalities in this group (thymol turbidity 2+ in 30% Takata reaction slightly positive in 10% and borderline bilirubin elevation in 2 cases) but there was no difference when compared to a similar follow up study in 69 cases with hepatitis outside pregnancy One case only had chemical evidence of probable mild liver disease Zondek and Brom-

berg are the only authors to report 2 cases of chronic hepatitis among their 23 survivors

Thus, incidence of residual liver disease after hepatitis during pregnancy is certainly not more frequent than in patients with hepatitis outside of pregnancy

Child survival from mothers with hepatitis during pregnancy Hepatitis during pregnancy induces a tendency toward abortion or premature delivery as does any other form of jaundice The mechanism is unknown There is no

correlation between premature delivery and duration of jaundice, serum bilirubin level or severity of clinical course (Ellegast 1954 a) Survival of the babies depends on their stage of maturity at birth and not on the mother's disease Table 9 summarizes the available data from the literature, excluding cases with therapeutic abortions

In the combined series of 383 cases 99 % of the babies died 34 % abortions, 55 % prematures and 10 % of babies delivered at term There were 204 % premature deliveries (196 % in the European and 279 % in the Mediterranean series) Of all prematures 136 % died in the European and 417 % in the Mediterranean collective series

Harnack and Martini report 20 women who had hepatitis 1 to 7 months before conception and were not jaundiced during pregnancy There were 2 abortions and 1 premature delivery in this series

A follow up study in children born of mothers with infectious hepatitis is reported by Ellegast et al (1954 c) Only 1 child was transiently jaundiced on the 7th day for 1 week, the others did not show any evidence of liver disease

Transplacental infection with hepatitis virus and incidence of malformation in babies of mothers with hepatitis during pregnancy The problem of transplacental infection of the embryo or baby with the hepatitis virus has been extensively discussed by several authors (Stokes et al, von Harnack and Martini Ellegast et al 1954 c, Mansell, Dorfler 1957 and

Dorfler 1958) The evidence for its occurrence is very meager

Hepatitis in babies within their first two months of life does occur, but their mothers are but exceptionally jaundiced during pregnancy On the other hand, practically none of the babies of mothers with clear-cut hepatitis during pregnancy have hepatitis Ellegast et al theorise that the babies are protected by the antibodies of their mothers, and that infection would only occur when viremia in the mother is present shortly before delivery, i.e. before the formation of antibodies

Dorfler in 1958 collected the available reports on the incidence of malformations Among 528 cases of hepatitis during pregnancy he found 19 malformations (reported in Harnack and Martini, Ellegast et al 1954 c, Bickenbach, Mansell) This incidence of 3.5 % is not significantly different from the 'normally' expected malformation rate of 2.4 % Not included in Dorfler's review are 2 malformations reported by Ingerslev and Teilmann and 1 case reported by Cahill, all 3 with hepatitis in the second half of pregnancy Most of these mothers were ill with hepatitis during the last trimester of gestation, which would not account for the malformations in their babies Kellog and Wesp observed 1 woman with hepatitis one month before conception who then delivered a monster A causal relationship is possible in this case

Thus transplacental infection with hepatitis virus and malformation of babies has not been completely ruled out but — if it does occur — appears to be extremely rare

Jaundice in liver cirrhosis during pregnancy

Cirrhosis of the liver and pregnancy is a very rare coincidence. Most cases of cirrhosis in women are seen past the childbearing age (Burslem et al). In young women with cirrhosis fertilisation is frequently precluded by the high incidence of amenorrhoea, oligomenorrhoea and non-ovulatory cycling (Labby).

At least 31 patients with cirrhosis of the liver and pregnancy are reported in the literature (Scaglione 1923 Kraul 1927 Hesselune 1930 Tenney and King 1933, Ashton 1934 Lascano and Pereyra 1936 Javert and Morrison 1951 3 cases Burslem et al 1952 2 cases Puyo 1953 2 cases Mack et al 1953 2 cases Slater 1954 Bearn et al 1956 2 cases Abrams 1956 Enrie et al 1957 Adno 1957 Nabriski et al 1958 2 cases Labby 1960 Moore and Hughes 1960 3 cases, O'Leary and Bepko 1962 Bennet et al 1963 Slaughter and Krantz 1963 2 cases). The age ranges from 24 to 42 years in these patients. Five underwent 2 pregnancies after cirrhosis had been documented (Burslem et al case 2, Abrams Nabriski et al case 2 Moore and Hughes case 3 Slaughter and Krantz case 1).

Not all cases are reported with enough details to allow for an exact tabulation. In at least 10 of the 31 patients there were no signs of deterioration of liver function during pregnancy (Kraul Tenney and King Burslem et al both cases Bearn et al both cases Adno Nabriski et al case 2 Moore and Hughes case 1 Slaughter and Krantz case 2). An 11th case had no symp-

ptoms during the course of gestation and massive but controllable uterine bleeding during delivery (Ashton). At least 7 of these 11 patients had jaundice, ascites and/or hematemesis prior to the reported asymptomatic gestation.

One patient developed ascites during pregnancy (Abrams) and in another pre-existing ascites increased (Hesselune). A total of 8 patients were jaundiced during pregnancy. In 4 pre-existing jaundice became more intense (Slater, Labby, Moore and Hughes cases 2 and 3) and in 3 jaundice developed during the last trimester of pregnancy. In 2 of these jaundice persisted for several months post partum (Puyo) and the third patient died 5 days after delivery in hepatic coma (Enrie et al). In the 8th patient jaundice decreased towards term (Bennet et al).

Only 4 patients developed hematemesis during pregnancy. In one it started in the 20th week of gestation, could not be controlled and the patient died (Lascano and Pereyra). In a second case hematemesis occurred during the 5th month and was successfully stopped by performing a porto-caval shunt (O'Leary and Bepko). The third case had hematemesis near term and ceased to bleed after a Caesarian section (Nabriski et al case 1). In the 4th case hematemesis started during labor and the patient exsanguinated (Scaglione).

There are a total of 4 deaths attributable to a complication of cirrhosis during pregnancy. 2 are due to hematemesis (in 1923 and 1936), 1 due to hepatic coma and a fourth case report gives no details (Javert and Morrison). In addition there were 2 late deaths. 1 six

months post partum from the sequelae of a splenectomy (Ashron) and 1 a year after delivery from liver failure (Slater)

Only 5 of the 36 children in this series died 2 still births in the 20th week (Lascano and Perceva) and near term (Bennet et al), 1 of congenital abnormalities (Moore and Hughes case 3) and 2 during delivery (Mack et al case 2, Slaughter and Krantz case 1)

Five patients had porto systemic shunts performed prior to the reported pregnancy 2 porto-caval shunts (Bearn et al case 24, Abrams) and 3 spleno-renal shunts (Adno, Labby, Slaughter and Krantz case 2) In all 5 there were no complications during gestation

In addition to these 31 patients with Laennec's or postnecrotic cirrhosis 3 pregnant patients with primary biliary cirrhosis are reported by Ahrens et al They suffered no ill effect from pregnancy

In general therefore, pregnancy is surprisingly well tolerated in a woman with cirrhosis of the liver It is probable that patients with the more severe forms of cirrhosis do not become pregnant and that the majority of cirrhotics with pregnancy represent a selection of benign cases

Drug-induced intrahepatic cholestasis during pregnancy

Of the long list of drugs capable of producing intrahepatic cholestasis (Dolic and Martini) chlorpromazine is the one most frequently used during pregnancy It has been advocated for the treatment of hyperemesis gravidarum by Moyer et al and by Benaron et al No cases of jaundice occurred in their

78 and 158 pregnancies respectively In the series of Stacey et al 8 of 170 non-pregnant patients (4.8%) developed icterus Low and Peel collected 10 single case reports of chlorpromazine jaundice during pregnancy

Chlorpromazine jaundice during pregnancy, whether short lasting or chronic, has no ill effect on the child Clinically it may simulate intrahepatic cholestasis of pregnancy during the icteric stage It usually starts much earlier because the drug is given for hyperemesis during the first trimester and usually clears well before delivery Onset of chlorpromazine jaundice is within 4 weeks after the drug has been started There is a prodromal phase of 4 to 5 days' duration, usually acute with malaise, fever, chills, nausea mild abdominal pains, myalgias and occasionally skin rashes Itching may precede jaundice (Werther and Korelitz, Sherlock) These prodromal symptoms are similar to those seen in viral hepatitis and will readily distinguish drug-induced cholestasis from intrahepatic cholestasis of pregnancy, in which there are no prodromata except pruritus

On occasion chlorpromazine jaundice may become chronic, and the question whether pregnancy may induce chronicity is unsettled Read et al collected 22 cases with chlorpromazine jaundice of more than 3 months duration Three of these started during pregnancy and include the case with the longest duration on record, i.e. 3 years This woman (case 1 of Read et al) was given chlorpromazine 75 mg daily during 8 days for hyperemesis and developed intrahepatic cholestasis with a maximum serum bilirubin of 21.5 mg per 100 ml

an alkaline phosphatase of 256 King—Armstrong units and a serum cholesterol of 1,120 mg per 100 ml. Later xanthomatosis appeared and the clinical picture resembled primary biliary cirrhosis. The jaundice cleared after 3 years and the xanthomas were fading after 4 years. A case of 10 months duration is recorded by Gebhardt et al and one of 7 months duration by Stacey et al. A fourth case of chronic chlorpromazine jaundice starting during pregnancy (not contained in Read's et al's review) is reported by Love and Peel with a duration of 6 months. All patients recovered completely. The longest duration of icterus in non pregnant subjects has been 9 and 13 months, with jaundice of less than 7 months duration in all the others.

Drug induced intrahepatic cholestasis, clinically and histologically of the chlorpromazine type has also been described after nitrofurantoin (Furodantin®) in an elderly female (Ermaelsteen and Williams) and recently in a pregnant woman (Goldstein and Contino). In both instances pyelonephritis for which the drug was given was severe but responded rapidly to treatment, whereas jaundice persisted for some weeks.

Obstructive jaundice due to choledocholithiasis during pregnancy

Jaundice due to common duct obstruction by gallstones is rare during pregnancy. In our collective series on jaundice during pregnancy there are only 27 cases recorded (table 4). Nine of these are contained in a single paper which casts some doubt on the diagnostic ac-

curacy. The rarity of this association even provokes single case reports (Rusmann 1909 and 1910, Brocq et al, Alex).

Gallstones have been said to occur more frequently in women with previous pregnancies than in childless women (Horn). Potter noted large atonic gall bladders with thick, tarry and viscous bile in three fourth of 390 women undergoing Caesarian sections. However, this did not cause stone formation. Large et al examined 352 pregnant women with cholecystographies and found gallstones in only 11. Furthermore, a large collective autopsy statistic by Robertson and Dochat showed that of 14,016 women with gallstones at autopsy, 79.6% had been previously pregnant but the incidence of previous pregnancies was nearly identical (79.2%) in autopsies of females without gallstones.

Jaundice during pregnancy due to an adenocarcinoma of the common duct has been noted twice (Caroli et al 1954, Vincent).

Effect of pregnancy in chronic idiopathic hyperbilirubinemias (Dubin—Johnson syndrome, Roter syndrome, Gilbert—Meulengracht syndrome)

In a review in 1958 Dubin collected 53 cases of chronic idiopathic jaundice (Dubin—Johnson syndrome). Nine of the 53 were women and 7 of the 9 underwent one or several pregnancies. (Dubin 1958 cases 16, 18 and 22, Klayman and Efrati, John and Knudtson, Tamaki, Carfagno). Pregnancy had no effect on the clinical course in one case precipitated an attack of jaundice in 2

months post partum from the sequelae of a splenectomy (Ashton) and 1 a year after delivery from liver failure (Slater)

Only 5 of the 36 children in this series died 2 still births in the 20th week (Lascano and Pereyra) and near term (Bennet et al), 1 of congenital abnormalities (Moore and Hughes case 3) and 2 during delivery (Mack et al case 2, Slaughter and Krantz case 1)

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hemolytic jaundice will practically never pose a diagnostic problem

Rare causes of jaundice during pregnancy

Of course, any disease capable of producing jaundice may once in a while be encountered during pregnancy, such as echinococcus of the liver (Jaer and Morrison, Synodinos et al), tuberculous of the liver (Earle et al) Weil's disease (Vincent), diffuse liver metastasis (Vincent) and leukemia (Javert and Morrison). A differential diagnosis of jaundice per se is beyond the scope of this paper

Jaundice in severe pyelonephritis during pregnancy

Before the advent of antibiotics pyelonephritis with jaundice was not infrequently seen during pregnancy (Decaudin). Jaundice was probably due to overwhelming septicemia. Toxic hemolysis may play an additional role (Rumbach).

Lepage in his thesis in 1934 collected 27 cases from the literature and added 4 of his own, calling the syndrome pyelonephritide gravidito-toxique. In our collected series from the literature since 1940 only 5 of 456 cases of jaundice during pregnancy were due to pyelonephritis (Table 5). Sheehan's autopsy series contains 12 pregnant women dying from pyelonephritis, 3 of which had mild jaundice during the last few days of life. The livers showed only mild non-specific changes.

This entity has now become rare. Pyelonephritis can be treated today before it progresses to a jaundiced stage. Effective treatment of pyelonephritis during pregnancy has however created

a new hazard: jaundice in pyelonephritis of pregnant women due to drug toxicity (see next paragraph)

Jaundice during pregnancy due to toxicity of drugs used in treatment of pyelonephritis (Tetracycline toxicity)

Schultz et al reported 6 autopsy cases of pregnant women treated for pyelonephritis with extremely high doses of intravenous tetracycline (24 to 40 gm per day). The patients became jaundiced 3 to 5 days after treatment was started with bilirubin levels of 5 to 12 mg per 100 ml, alkaline phosphatase of 9 to 44 King-Armstrong units, positive cephalin flocculations and mild elevation of serum glutamic oxalacetic transaminases (70-170 units). The patients died within 5 to 13 days. The livers showed diffuse fatty metamorphosis.

One of the two cases reported by Kahil et al and the case of Lewis et al, both diagnosed as acute fatty metamorphosis of pregnancy, had also received high doses of tetracycline and streptomycin for severe pyelonephritis. At post mortem examination fatty metamorphosis was diffuse and the characteristic rim of intact liver cells in the periphery of all lobules seen in acute fatty metamorphosis of pregnancy was not present.

Whalley et al reported 5 similar cases with survival in four. Their treatment for pyelonephritis consisted of intravenous tetracycline 1.5-2 gm per day and intramuscular streptomycin 1 gm per day for 4-17 days. All had renal failure and an associated pancreatitis. Fatty metamorphosis was predominantly centrilobular in the liver biopsies of the 4

and aggravated pre-existing jaundice in 4 Icterus became gradually more intense, reached its height in the third trimester and waned or disappeared completely after delivery of the child. Clinically the disease may simulate extra-hepatic biliary obstruction, but itching is absent and the alkaline phosphatase is usually within normal limits. Diagnosis may be suspected from the history of chronic jaundice without severe impairment of health and is easily verified by liver biopsy.

A similar syndrome but with normal liver biopsy findings has been described by Rotor. It is not clear at present, whether this is a disorder by itself or a milder form of the Dubin—Johnson syndrome. The patients with the *Rotor syndrome* have permanent mild jaundice but are otherwise asymptomatic. One woman reported in the literature underwent three pregnancies, each time with a decrease in the intensity of jaundice (Haverback and Wirtschafter, case 2).

In both the Rotor and the Dubin—Johnson syndrome the direct reacting serum bilirubin fraction is elevated. A normal direct reacting bilirubin with a mild elevation of the indirect-reacting fraction occurs in the *Gilbert—Meulengracht syndrome*. These patients are asymptomatic except for some fatigue (Foulk et al). The syndrome occurs less frequently in females than in males. Stress, exercise and upper respiratory infections are said to increase jaundice temporarily. The effect of pregnancy on bilirubin levels in these patients has not been studied. Meulengracht reports two women who were free of jaundice during gestation.

Hemolytic jaundice during pregnancy
Hemolytic jaundice during pregnancy is very rare (Zachariae). It may be observed after incompatible blood transfusions. Jaundice observed in severe eclampsia and in overwhelming infections (pyelonephritis) has been classified by Sheehan as hemolytic.

Megaloblastic anemia of pregnancy
may cause severe anemia, but rarely visible jaundice. In most cases it is due to dietary folic acid deficiency (Sanders). Occasionally vitamin B₁₂ deficiency may be encountered, but is, if present, usually combined with folic acid deficiency (Lowenstein et al). *Hemolytic anemia of pregnancy* (without demonstrable cause other than pregnancy itself) may produce jaundice, but is extremely rare (Dameshek and Schwartz, Crismer).

More often, hemolytic jaundice during pregnancy is caused by an exacerbation of a pre-existing chronic hemolytic state (Bromberg et al). This may occur in congenital spherocytosis (Rimbach and Beickert) and in some hemoglobinopathies, especially in S—C, S—S and C—C disease (Fouche and Switzer, Curtis). Jaundice may become marked in some instances. The combination of gestation and hemoglobinopathy is hazardous for both mother and child.

A rare case may have a combined etiology, such as megaloblastic anemia of pregnancy superimposed on congenital spherocytosis (Schneider and Frahm).

A classification of hemolytic anemias during pregnancy is given in the paper of Zachariae.

During pregnancy hemolysis presents as marked anemia and an accompanying

hemolytic jaundice will practically never pose a diagnostic problem

Rare causes of jaundice during pregnancy

Of course, any disease capable of producing jaundice may once in a while be encountered during pregnancy, such as echinococcus of the liver (Javert and Morrison Synodinos et al), tuberculoma of the liver (Ennle et al), Weil's disease (Vincent), diffuse liver metastasis (Vincent) and leukemia (Javert and Morrison). A differential diagnosis of jaundice per se is beyond the scope of this paper.

Jaundice in severe pyelonephritis during pregnancy

Before the advent of antibiotics pyelonephritis with jaundice was not infrequently seen during pregnancy (Decaudin). Jaundice was probably due to overwhelming septicemia. Toxic hemolysis may play an additional role (Rimbach).

Lepage in his thesis in 1934 collected 27 cases from the literature and added 4 of his own calling the syndrome *pyelonephrite dite gravidotoxique*. In our collected series from the literature since 1940 only 5 of 436 cases of jaundice during pregnancy were due to pyelonephritis (Table 5). Sheehan's autopsy series contains 12 pregnant women dying from pyelonephritis, 3 of which had mild jaundice during the last few days of life. The livers showed only mild non-specific changes.

This entity has now become rare. Pyelonephritis can be treated today before it progresses to a jaundiced stage. Effective treatment of pyelonephritis during pregnancy has however created

a new hazard—jaundice in pyelonephritis of pregnant women due to drug toxicity (see next paragraph).

Jaundice during pregnancy due to toxicity of drugs used in treatment of pyelonephritis (Tetracycline toxicity)

Schultz et al reported 6 autopsy cases of pregnant women treated for pyelonephritis with extremely high doses of intravenous tetracycline (24 to 40 gm per day). The patients became jaundiced 3 to 5 days after treatment was started, with bilirubin levels of 5 to 12 mg per 100 ml, alkaline phosphatase of 9 to 44 King—Armstrong units, positive cephalin flocculations and mild elevation of serum glutamic oxalacetic transaminases (70—170 units). The patients died within 5 to 13 days. The livers showed diffuse fatty metamorphosis.

One of the two cases reported by Kahil et al and the case of Lewis et al both diagnosed as acute fatty metamorphosis of pregnancy had also received high doses of tetracycline and streptomycin for severe pyelonephritis. At post mortem examination fatty metamorphosis was diffuse and the characteristic rim of intact liver cells in the periphery of all lobules seen in acute fatty metamorphosis of pregnancy was not present.

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This observation implicates tetracycline as the responsible toxic agent. It is curious that this type of tetracycline toxicity has never been observed outside pregnancy and that the liver lesion is nearly indistinguishable from acute fatty metamorphosis of pregnancy, which does also not occur outside of gestation.

Nitrofurantoin (Furodantin®)-induced intrahepatic cholestasis is another but benign disorder occurring in the treatment of pyelonephritis. It occurs also in non pregnant subjects and has been discussed in the chapter on drug-induced cholestasis.

Jaundice due to delayed chloroform poisoning

This form of jaundice occurs only after delivery and is furthermore mainly of historic interest. It shall be mentioned here because it rarely occurred in non obstetric patients.

Sheehan reported 14 autopsy cases in 1940, which fell into 3 groups. Three patients apparently healthy aside from

pregnancy had received an overdose of chloroform and died within 2 days without evidence of hepatic dysfunction. At autopsy there were isolated cell lesions involving more than half of the hepatocytes in the middle and center of the lobules. Nine patients with prolonged labor before chloroform narcosis developed jaundice, fever and coma on the second postpartum day and died 2 to 4 days later. The striking finding at autopsy was a band of mid zonal necrosis. Two patients with severe hyperemesis before chloroform narcosis had central necrosis at postmortem examination.

Intrahepatic cholestasis of pregnancy

Intrahepatic cholestasis of pregnancy is — after viral hepatitis — the second most frequent cause of jaundice during pregnancy. It accounts for 20.6% of the 436 cases with jaundice listed in Table 4. It has a marked tendency to recur in subsequent pregnancies, but is entirely benign to both mother and child. The disease is characterized by marked pruritus followed by mild jaundice with no other prodromal symptoms and no impairment of general well being. Jaundice appears in the majority of cases after the 22nd week of gestation but may start as early as the 7th week in some patients. Jaundice and pruritus disappear rapidly after spontaneous or induced delivery. Biochemically the disorder presents the features of 'obstructive jaundice' with no evidence of parenchymal liver cell damage. Bile flow in the extrahepatic biliary passages is unimpaired. Liver biopsies show minimal intrahepatic cholestasis.

Details on clinical, biochemical and

histological features of this disease will be discussed in the chapter on recurrent intrahepatic cholestasis of pregnancy

Acute fatty metamorphosis of pregnancy

In 1940 Sheehan delineated from true acute yellow atrophy of the liver due to fulminating viral hepatitis a new entity 'obstetric acute yellow atrophy', which was later termed 'acute fatty metamorphosis of pregnancy' by Ober and Lecompte. Its etiology is unknown, but it occurs only in pregnant females. This entity presents a clear cut and diagnostic histological picture of the liver, but is clinically nearly indistinguishable from fulminant viral hepatitis.

Histologically it consists of a gross fatty change which starts in the center of the acini and involves finally the entire liver lobule with the exception of a sharply defined rim of normal liver cells around the portal tracts. There is a striking absence of necrosis and of inflammatory reaction. Only occasionally a very light infiltration of small round cells may be seen throughout the fatty area. The portal tracts show no abnormalities (Sheehan 1940). Bile thrombi may be present in the center of the lobules (Moore). Compared to fulminant hepatitis there is a striking lack of post mortem autolysis. In the one survivor followed by serial liver biopsies (Whitacre and Fang) resitution of liver cells began from the remaining rim of normal cells in the periphery of the lobules and then progressed towards their center.

Some confusion exists in the literature resulting from the inclusion of cases with diffuse fatty metamorphosis without a peripheral rim of intact liver cells. Such

cases probably do not belong to the entity described by Sheehan. The case reported by Lewis et al for instance is a typical example of tetracycline toxicity in pyelonephritis during pregnancy. Tetracycline toxicity must be implicated also in case 1 reported by Kahil et al.

In the literature 40 cases of acute fatty metamorphosis of pregnancy have been reported (Stander and Cadden 1934, Cullinan 1936 cases A and B, Sheehan 1940 6 cases, Whitacre and Fang 1942, Nixon et al 1947 case 20, Barry and O'Dwyer 1955 case 9, Ober and Lecompte 1955 3 cases, Moore 1955, Moore 1956 4 cases, Mason 1958, Dyson 1959, Nardone et al 1961, Sheehan 1961 2 cases, Bruno and Ober 1962 5 cases, Peters et al 1963 7 cases, clinicopathologic conference 1963, Siegler and Heyser 1963, Kahil et al 1964 case 2). Not included in this review are 11 additional cases: 6 because the histological findings are different or inconclusive (Biens and Espinola 1937, Dill 1950 cases 1, 2 and 6, Lewis et al 1963, Kahil et al 1964 case 1) and 5 in which the patients recovered and no liver biopsies were performed (Duncan and McLachlan 1933, Taylor 1952, Pinnoli 1957, Edwards 1960, Sheehan 1961).

Acute fatty metamorphosis of pregnancy has been noted in the age range of 16 (Ober and Lecompte case 3) to 42 years (Nardone et al, Dyson). In the majority of cases it occurred during the first pregnancy. In only 3 of 40 cases it was observed after the second gestation during the third pregnancy in the CPC case during the sixth in Moore's case 2 and during the 8th in case 2 of Nardone et al.

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Of the 40 acceptable cases only 6 women survived Two of these are reported in abstract form (Peters et al) and in a third no details are given (Sieglar and Keyser) Of the remaining 3 cases 2 were delivered by an early Caesarian section (Whitacre and Fang, Moore case 2) and in one the first symptom occurred after delivery (Moore case 3) Another 5 cases with recovery but without histological proof of the diagnosis have been mentioned above

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Acute fatty metamorphosis of pregnancy is thus a rare but distinct entity not occurring outside of pregnancy. It carries a grave prognostic outlook but is potentially reversible. As rapid deterioration sets in always after delivery and as both women delivered by Caesarian section survived it appears reasonable to propose as an only chance for both mother and child a therapeutic Caesarian section as early as possible after onset of symptoms.

Jaundice in hyperemesis gravidarum

Jaundice in hyperemesis gravidarum is rare, usually mild and does not imply a poor prognostic outlook. In a thesis in 1926 Ferru could collect only 22 cases from the literature, one of which died.

Of 51 patients with hyperemesis reported by Verhage 15 had a slight serum bilirubin elevation, but only a few were frankly jaundiced. Urinary urobilinogen was increased in most and urine bilirubin was present in about one third. Of Millars 120 patients 5 were jaundiced. In Klier's series of 119 patients 12 had elevated serum bilirubin levels ranging from 1.9 to 8.6 mg per 100 ml. All had proteinuria and ketonuria. The cadmium reaction was negative in all and the thymol turbidity positive in one. A further 7 patients without jaundice had bilirubin and urobilin present in their urines. All patients recovered. Of Herold's 25 patients 8 had a serum bilirubin of more than 1 mg per 100 ml, with 2 peak values of 4.4 and 6.7 mg per 100 ml. Bilirubinuria was present in those with a serum bilirubin of more than 1.5 mg per 100 ml. Direct reacting bilirubin was positive in 19 and the galactose tolerance test impaired in 16. Thorling reports 6 patients with hyperemesis and jaundice. Five had associated or preceding pruritus, which ran parallel with the vomiting in three. Three patients had an elevated alkaline phosphatase. The thymol turbidity was normal in all. Both serum transaminases may be increased in the more severe cases. SGOT up to 82 units, SGPT up to 283 units (Durst and Strauss).

No liver biopsies have been performed in this disease. Sheehan reported in

Onset of symptoms was in 30th week in two cases (Moore case 1 and 2), in the 31st week in 1 (Ober and Lecompte case 1), in the 34th in 2 (Moore case 4, CPC case) and in the others between the 36th and 40th week. The disease starts rather suddenly with severe and persistent vomiting, occasionally accompanied by abdominal pains, followed in a few days by jaundice. Tachycardia of between 100 and 130 is regularly present, but there is no fever. The symptoms then progress rapidly, the jaundice becomes intense, the patient somnolent, the vomiting assumes a coffee ground aspect and frank hematemesis may supervene. Severe headaches are noted in some patients. The laboratory features are consistent with an obstructive jaundice, except that urinary bilirubin may not be present until terminally. The highest recorded serum bilirubin levels were 12.7 (Whitacre and Fang), 16.8 (Moore case 3) and 26 mg (CPC case) per 100 ml, all others being below 10 mg per 100 ml. The alkaline phosphatase is increased to levels up to 54 King-Armstrong units (Moore). Serum transaminases have been recorded in only 2 cases with values between 100 and 300 units (CPC, Kahil et al). Thymol turbidity and cephalin flocculation tests are normal with one exception (Kahil et al). Prothrombin time is markedly decreased. White cell counts ranged from 19,200 to 32,000 per cu mm in the 7 recorded cases (Barry and O'Dwyer, Ober and Lecompte, Kahil et al, CPC). A few patients had marked hypoglycemic episodes (Standen and Cadden, Whitacre and Fang, Kahil et al). In about half of the cases oliguria sets in, followed by

azotemia. A disproportionate rise of urea acid levels has been noted by Sheehan, and a disproportionate rise of serum creatinine in the CPC case. Others have seen a drop of the previously elevated blood urea nitrogen to abnormally low levels just before death (Barry and O'Dwyer, Mason, CPC).

The usual course is steadily downhill. Premature labor sets in, the woman delivers a stillborn child and then lapses into deep coma, occasionally accompanied by convulsions. High fevers may now occur and the patient dies shortly thereafter. The duration from the first symptom until death may vary between 3 days and 4 weeks and is usually between 1 and 2 weeks. The duration from delivery to death is usually 2 to 4 days, but may range from one hour (Ober and Lecompte case 3) to a maximum of 7 days (Moore case 4).

Some minor exceptions to this typical course may occur. Two cases died undelivered before ever having been jaundiced (Dyson, Nardone et al), another died undelivered during the icteric stage (Cullinan, case A). In 2 others the first symptom appeared only after delivery (case 20 of Nixon et al, case 3 of Moore) and in one jaundice began 4 days post partum (Ober and Lecompte, case 2). In two cases pruritus was present (Ober and Lecompte case 1, CPC case). Four patients developed ascites (Ober and Lecompte case 1, Moore case 3, Whitacre and Fang, Kahil et al case 2). Two patients bled profusely during delivery (Mason, CPC case). At autopsy a clinically unsuspected acute hemorrhagic pancreatitis was found in 3 of Bruno and Ober's and in 4 of Peters et

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1939 19 autopsy cases of patients dying from hyperemesis. Those with a duration of symptoms of more than 6 weeks were slightly jaundiced. Thirteen had cerebral symptoms prior to death which were interpreted as Wernicke's encephalopathy. Histologically the liver was normal in 7 and showed in 12 a slight fatty infiltration which was considered insignificant and did not lead to necrosis. A specific liver lesion has not been found and most reported histological changes could also be due to starvation and avitaminosis.

The incidence of hyperemesis gravidarum appears to be on the decline. Guttmacher reports that in 1937 one in every 150 pregnant patients had to be hospitalised for hyperemesis, while in 1957 the figure was only 1 in 974. In reviews on jaundice during pregnancy no series since 1940 reports more than 6 patients with jaundice due to hyperemesis (see Table 4). The clinical problem encountered will be mainly one of differential diagnosis from the prodromal phase of viral hepatitis, which should not be too difficult when tests such as serum transaminases and thymol turbidity are employed.

Jaundice in hyperemesis may recur in successive pregnancies.

Jaundice in vomiting of late pregnancy

Apart from hyperemesis gravidarum which occurs in the first trimester and almost always clears spontaneously by the 12th week of gestation there is a syndrome of "pernicious vomiting in late pregnancy" occurring usually between the 33rd and 37th week. Almost always an underlying cause like pyelonephritis

other infections or pre-eclampsia is found. Sheehan reports in autopsy series of 16 such cases, of which 4 had a serum bilirubin elevation to maximally 2 mg per 100 ml in the last few days of life. The livers showed histologically no significant changes or a slight fatty infiltration.

Clinically this syndrome never presents as a case of jaundice. The term should best be abandoned, because rarely does vomiting appear to be "idiopathic".

Jaundice in toxemia of pregnancy

Toxemia of pregnancy is accompanied by an increased incidence of abnormal so-called liver function tests, but liver function on the whole is not impaired. Among 44 patients Dieckmann et al found in 51 % a positive cephalin flocculation and in 43 % a positive thymol turbidity test. A slight increase in bromsulphalein retention was noted by Christhilf and Bonsnes. Serum alkaline phosphatase was elevated in 75 % of Mukherjee's 100 toxemic patients, compared to a 28 % incidence in his normal pregnant controls. The observed range was 12.5 to 16.5 King-Armstrong units in mild and 13.4 to 29.5 units in severe cases. Alkaline phosphatase closely parallels the clinical course in eclampsia and increases with the number of convulsions. Serum glutamic oxaloacetic transaminase and lactic dehydrogenase are often elevated in the more severe cases (Kubli). Within the first 36 hours after admission SGOT was elevated in all of Crisp et al's 64 patients with values above 100 units (maximum 244 units) in all severe cases. A rapid fall in SGOT indicated a good prognosis.

Jaundice is rare in toxemia. occurs

late in the course and often suggests a grave prognosis. The bilirubin increase in 17% of Dieckmann et al's 44 patients was only slight. Of Verhage's 96 patients with toxemia only 11 had icterus, 2 of which died. In Sheehan's autopsy series of 90 cases only 10 were jaundiced. Sheehan believes the icterus to be due to hemolysis, as his jaundiced patients had hemoglobinuria during life and hemoglobin casts in the kidney tubules at autopsy.

Histological liver lesions so striking at autopsy, are absent in mild and even in some far advanced cases on liver biopsies (Ingerslev and Teilum III). Of 15 severe cases with convulsions only 5 showed definite liver lesions in Antia et al's biopsy series. In Sheehan's opinion histological changes occur only during the last two days of life.

The liver lesions reflect the basic vascular disorder. Characteristic findings consist of fibrin thrombi in the sinusoids of the periportal zone. Similar thrombi occur in other organs. In the liver they obstruct sinusoidal blood flow and cause sinusoidal dilatation which leads to necrosis of clusters of hepatic cells (often wrongly called fibrinoid necrosis) and finally to hemorrhagic necrosis of the periportal zone. If the patient has been in shock, centrilobular necrosis may be superimposed and the areas of hemorrhagic necrosis may become confluent and form large blood pools. An inflammatory reaction is characteristically absent. Liver lesions in eclampsia are therefore interpreted as a terminal event (Sheehan, Ingerslev and Teilum III, Dietel 1947, Dieckmann 1952, Antia et al 1958).

The rare case of jaundice due to toxemia of pregnancy will hardly present clinically as an icterus of unknown origin.

5) Indications for interruption of pregnancy because of jaundice

Interruption of pregnancy because of a disease causing jaundice during pregnancy is never indicated with the possible exception of acute fatty metamorphosis of pregnancy.

This statement will not be questioned when applied to chronic idiopathic hyperbilirubinaemias or to drug induced intrahepatic cholestasis and tetracycline toxicity. In the rare case of jaundice due to common duct obstruction surgery on the biliary tract can be performed during gestation. When jaundice of this kind develops after the 36th week surgery may be delayed until after delivery. Treatment in jaundice due to pyelonephritis with septicemia is directed at the infection.

Intrahepatic cholestasis of pregnancy is definitely not an indication to terminate pregnancy. Although an induced interruption of pregnancy will cure the disorder such a cure will occur in all cases after spontaneous delivery. Symptoms are never so distressing as to justify a shortening of the jaundiced period since pruritus can be effectively relieved by cholestyramine. Many interruptions have been performed especially in the recurrent form, but usually by physicians who were not familiar with this disease and its absolute and constant benignity.

An interruption of pregnancy has

1939 19 autopsy cases of patients dying from hyperemesis. Those with a duration of symptoms of more than 6 weeks were slightly jaundiced. Thirteen had cerebral symptoms prior to death which were interpreted as Wernicke's encephalopathy. Histologically the liver was normal in 7 and showed in 12 a slight fatty infiltration which was considered insignificant and did not lead to necrosis. A specific liver lesion has not been found and most reported histological changes could also be due to starvation and avitaminosis.

The incidence of hyperemesis gravidarum appears to be on the decline. Guttmacher reports that in 1937 one in every 150 pregnant patients had to be hospitalised for hyperemesis, while in 1957 the figure was only 1 in 974. In reviews on jaundice during pregnancy no series since 1940 reports more than 6 patients with jaundice due to hyperemesis (see Table 4). The clinical problem encountered will be mainly one of differential diagnosis from the prodromal phase of viral hepatitis, which should not be too difficult when tests such as serum transaminases and thymol turbidity are employed.

Jaundice in hyperemesis may recur in successive pregnancies.

Jaundice in vomiting of late pregnancy

Apart from hyperemesis gravidarum which occurs in the first trimester and almost always clears spontaneously by the 12th week of gestation there is a syndrome of "pernicious vomiting in late pregnancy" occurring usually between the 33rd and 37th week. Almost always an underlying cause like pyelonephritis

other infections or pre-eclampsia is found. Sheehan reports an autopsy series of 16 such cases, of which 4 had a serum bilirubin elevation to maximally 2 mg per 100 ml in the last few days of life. The livers showed histologically no significant changes or a slight fatty infiltration.

Clinically this syndrome never presents as a case of jaundice. The term should best be abandoned, because rarely does vomiting appear to be "idiopathic".

Jaundice in toxemia of pregnancy

Toxemia of pregnancy is accompanied by an increased incidence of abnormal so-called liver function tests, but liver function on the whole is not impaired. Among 44 patients Dieckmann et al found in 51 % a positive cephalin flocculation and in 43 % a positive thymol turbidity test. A slight increase in bromsulphalein retention was noted by Christliff and Bonsnes. Serum alkaline phosphatase was elevated in 75 % of Mukherjee's 100 toxemic patients compared to a 28 % incidence in his normal pregnant controls. The observed range was 12.5 to 16.5 King-Armstrong units in mild and 13.4 to 29.5 units in severe cases. Alkaline phosphatase closely parallels the clinical course in eclampsia and increases with the number of convulsions. Serum glutamic oxalacetic transaminase and lactic dehydrogenase are often elevated in the more severe cases (Kubli). Within the first 36 hours after admission SGOT was elevated in all of Crisp et al's 64 patients with values above 100 units (maximum 244 units) in all severe cases. A rapid fall in SGOT indicated a good prognosis.

Jaundice is rare in toxemia; occurs

late in the course and often suggests a grave prognosis. The bilirubin increase in 17 % of Dieckmann et al.'s 44 patients was only slight. Of Verhage's 96 patients with toxemia only 11 had icterus, 2 of which died. In Sheehan's autopsy series of 90 cases only 10 were jaundiced. Sheehan believes the icterus to be due to hemolysis, as his jaundiced patients had hemoglobinuria during life and hemoglobin casts in the kidney tubules at autopsy.

Histological liver lesions, so striking at autopsy, are absent in mild and even in some far advanced cases on liver biopsies (Ingerslev and Teilum III). Of 15 severe cases with convulsions only 5 showed definite liver lesions in Anzia et al.'s biopsy series. In Sheehan's opinion histological changes occur only during the last two days of life.

The liver lesions reflect the basic vascular disorder. Characteristic findings consist of fibrin thrombi in the sinusoids of the periportal zone. Similar thrombi occur in other organs. In the liver they obstruct sinusoidal blood flow and cause sinusoidal dilatation, which leads to necrosis of clusters of hepatic cells (often wrongly called fibrinoid necrosis) and finally to hemorrhagic necrosis of the periportal zone. If the patient has been in shock centrilobular necrosis may be superimposed and the areas of hemorrhagic necrosis may become confluent and form large blood pools. An inflammatory reaction is characteristically absent. Liver lesions in eclampsia are therefore interpreted as a terminal event (Sheehan, Ingerslev and Teilum III, Dietel 1947, Dieckmann 1952, Anzia et al. 1958).

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An interruption of pregnancy has

often been advocated in severe cases of viral hepatitis. As has already been pointed out in the respective chapter, no evidence exists that the course of viral hepatitis is affected by pregnancy itself. Pregnancy is influenced by viral hepatitis in a tendency to premature deliveries. However, spontaneous abortions in severe cases of hepatitis do not improve the course of the disease, and furthermore a turn to the worse has been observed after spontaneous abortions. In addition, any narcosis or surgery is very poorly tolerated in severe cases of hepatitis, which again is a strong point against a surgical termination of pregnancy.

The course of a patient with liver cirrhosis is generally not influenced by pregnancy. It has already been pointed out, that cirrhotic females with pregnancies present in a way a selection of cirrhotics with fairly good liver function, as in severe cases of cirrhosis fertilisation is rare. Pregnancy may possibly increase portal hypertension by an increase in intraabdominal pressure. In pregnant cirrhotics with hematemesis due to esophageal varices successful porto-caval shunts have been performed. The rare case in which liver function decompensates critically during pregnancy would probably have a poor prognostic outlook even without pregnancy. Furthermore, in such a case surgery is as poorly tolerated as in severe viral hepatitis. For all these reasons an induced termination of pregnancy appears not indicated in cirrhosis.

In rare cases of severe hyperemesis gravidarum or severe toxemia of pregnancy a termination of gestation may

become necessary. The indications for an interruption of pregnancy will be based on general obstetrical principles and will not be based on the presence or absence of jaundice. In hyperemesis gravidarum jaundice does not carry a grave prognosis. In toxemia of pregnancy jaundice occurs only in the severe cases and its presence may be an additional point for the obstetrician in favour of a termination of gestation. Valuable guides for the prognostic outlook in toxemia of pregnancy are the serum alkaline phosphatase and the serum transaminases.

Acute fatty metamorphosis of pregnancy is probably a valuable and furthermore the only indication to terminate pregnancy because of jaundice. In order to be successful a Caesarian section has to be performed very early in the course, which necessitates an early and correct diagnosis for which a liver biopsy is nearly indispensable. As performance, embedding and reading of a liver biopsy usually takes two days in the most favorable circumstances, it may be necessary to avoid this delay and to take a decision without a biopsy, based on the typical clinical symptoms and signs.

Interruption of pregnancy may be indicated in some hemoglobinopathies which are known to take a deleterious course under the influence of pregnancy (especially S—C, S—S and C—C disease) and in some hemolytic anemias during pregnancy not responding to medical treatment. In these instances the decision will be taken because of hemolysis and not because of jaundice. Jaundice if present is mild anyhow in these disorders.

III Recurrent jaundice during pregnancy

Clear definitions of the different diseases disguised under the term recurrent jaundice during pregnancy are hampered by the similarity of the clinical course between many of these disorders, which are nearly all benign as evidenced by their recurrence during successive pregnancies. Puj⁸ wrote in his thesis in 1953: *Les travaux publiés, malgré le nombre de cas rapportés et le nombre de données (clinique, biologique et histologique) souvent réunis, sont mal utilisables quand on désire se faire une idée des particularités de ces ictères.* Furthermore the majority of case reports are but sparsely documented with laboratory data and rarely verified by liver biopsies. The problem of delivering the pregnant patient usually takes precedence over the problem of investigating the cause of jaundice, especially as jaundice is mild in this syndrome and clears rapidly after delivery.

1) Historical note

Recurrent jaundice during pregnancy has to our knowledge first been noted by Hoffman in 1872 (une jeune femme laquelle à chaque grossesse devenait icterique peu de temps avant d'accoucher). The first case report giving some details was published by Ahlfeld in 1881. Up to 1910 15 cases were known in the medical literature. During the next 12

years only 1 case was published. Interest in this disease continued to be minor, with 11 cases reported between 1923 to 1935 and 5 cases reported from 1940 to 1948. The years 1950 to 1959 brought 30 new cases and the discovery of further patients promises to be profuse in the 1960's.

Many of the early authors concluded that in the case they described jaundice was in some way linked to the state of gestation. With the description of additional cases it became apparent that at least two thirds of all patients with recurrent jaundice during pregnancy had similar and typical symptoms (defined on page 36) and that they therefore must represent a definite disease entity. This disease, now called recurrent intrahepatic cholestasis of pregnancy, has been clearly and fully described by two Swedish authors, Stanborg in 1954 and Thorling in 1955. Both workers also defined the first attack, i.e. the non recurrent form of the same disease. The intrahepatic cholestasis mechanism has however been definitely expressed in a case report by Perreau in 1953 and was probably first alluded to by Boreel in 1924 at Van den Bergh's department. The first liver biopsies in this disease were performed by Ljunggren in 1956 and Gros in 1958.

The difficulties in clearly separating the different disorders causing recurrent

jaundice during pregnancy are best exemplified in the largest single series of cases (Perreau and Rouchy 1961). Of the 9 cases in the report only 7 conform to the diagnosis of recurrent intrahepatic cholestasis of pregnancy. Case II had histologically the features of cirrhosis of the liver and case III is an example of recurrent pruritus during pregnancy.

2) Recurrent intrahepatic cholestasis of pregnancy

Nomenclature

Recurrent jaundice of pregnancy has originally been used as a purely descriptive term, meaning the occurrence of jaundice during successive pregnancies. As more and more cases were described in the literature, the term assumed the meaning of a specific disease (Magnani 1924, Schwalm 1932, Perreau 1953). Finally, 'recurrent jaundice of pregnancy' was used by Svanborg in 1954 to include also the non recurrent form of the same disease (i.e. first attack in just one pregnancy). While most cases described under this heading are examples of intrahepatic cholestasis of pregnancy, other forms of jaundice during pregnancy may also present as recurrent jaundice during successive gestations (discussed under differential diagnosis of recurrent jaundice) which further confuses the issue. In Thorling's opinion the term "recurrent jaundice of pregnancy" designating a form of jaundice peculiar to pregnancy, has become redundant.

We share Thorling's opinion and reject the term recurrent jaundice of

pregnancy" as meaning a specific disease, but we propose to retain the term 'recurrent jaundice during pregnancy' as a purely descriptive one without etiological implications, just as the term "jaundice" alone is descriptive without implying a specific disease entity.

The disease formerly called 'recurrent jaundice of pregnancy' is in our opinion best termed recurrent 'intrahepatic cholestasis of pregnancy'. A multitude of other names have been used for its designation. The term most frequently employed in the older literature is 'icterus gravidarum' (Ahlfeld 1881, Becking 1896, Von den Velden 1904, Boreel 1924) or "Schwangerschaftsicterus" (Benedict 1902, Mayer 1906) and 'Graviditätsicterus' (Brauer 1903). However, the same term served also to include all forms of jaundice during pregnancy. The word "idiopathic" was then added, as in "idiopathic hepatopathy of pregnancy" (Ljunggren 1936), 'idiopathischer Schwangerschaftsicterus' (Eppinger 1937, Gros 1958, Wilken 1958) and 'idiopathic jaundice of pregnancy' (McAllister and Waddell 1962, King and Kerrins 1963). The etiology of the disease is certainly unknown and the term 'idiopathic' is at present justified, but "acute fatty metamorphosis of pregnancy" is also idiopathic and does also occur only in pregnant women (Moore 1963). 'Benign jaundice of pregnancy' (Orellana et al 1961) and 'Hepatic benigne de la grossesse' (Caroli et al) appear correct but not precise enough.

Attempts at a pathogenetic description are expressed in the next group of terms: 'Hepatosi' (Sheehan 1961) or 'obstetric hepatosis' (Ilkonen 1964) ex

TABLE 10 Recurrent jaundice during pregnancy Classification of cases used in review of literature

Category	Description	Number of cases	Tables
I	Recurrent intrahepatic cholestasis of pregnancy with liver biopsies	23	Table 11
	I A) with laboratory data and details on clinical course	III	Tables 13 16
	I B) without further details	5	Table 18
II	Probable recurrent intrahepatic cholestasis of pregnancy with sufficient laboratory data but without liver biopsies	20	Table 17
	II A) with details on clinical course	13	Table 14
	II B) without further details	7	Table 19
III	Recurrent jaundice during pregnancy with details on clinical course No biopsies no or scarce laboratory data	12	Table 13
IV	Recurrent jaundice during pregnancy number of gestations mentioned only	26	Table 20
V	Recurrent jaundice during pregnancy no details reported	23	Table 21
VI	Recurrent jaundice during pregnancy sufficient details reported to exclude intrahepatic cholestasis of pregnancy (liver biopsy in 6 cases)	28	—
	Total cases of recurrent jaundice during pregnancy	132	
VII	Non recurrent jaundice during pregnancy considered to represent possibly intrahepatic cholestasis by original author or by reviewer	267	Table 22
	VII A) cases with liver biopsy	33	Table 12

References are given in the respective tables.

cludes an infectious agent but has been applied by others to jaundice seen in hyperemesis and eclampsia (Gros 1958)

Hepatitis tox gravidique (Caroli et al 1954) might as well be used in eclampsia with jaundice and is therefore non specific. Endogenous hepatotoxaemia of pregnancy (Thorling 1955) has the same disadvantage as idiopathic and could also include acute fatty metamorphosis of pregnancy. Furthermore the term calls for a counterpart exogenous hepatotoxaemia of pregnancy which was used by Thorling for jaundice during pyelonephritis in gestation but

which might also be employed in other septicemias, in chloroform poisoning, or in drug induced jaundice

In a designation for the disease the intrahepatic cholestasis mechanism was first alluded to by Caroli et al (1954) in the term *forme dite cholestatique pure des hepatites icterogenes de la grossesse* later changed to *ictere cholestatique de la grossesse* by Perreau and Rouchy (1961). Intrahepatic cholestasis of pregnancy finally has been used by Haemmerli and Wyss for the presentation of their first 5 cases (Annual Meeting of the Swiss and German Gastro-

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We share Thorling's opinion and reject the term 'recurrent jaundice of

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Attempts at a pathogenetic description are expressed in the next group of terms: Hepatosis (Sheehan 1961) or obstetric hepatosis (Honon 1964) ex

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Category	Description	Number of cases	Tables
I	Recurrent <i>intrahepatic</i> cholestasis of pregnancy with liver biopsies	23	Table 11
	I A) with laboratory data and details on clinical course	18	Tables 13 16
	I B) without further details	5	Table 18
II	Probable recurrent <i>intrahepatic</i> cholestasis of pregnancy with sufficient laboratory data but without liver biopsies	20	Table 17
	II A) with details on clinical course	15	Table 14
	II B) without further details	5	Table 19
III	Recurrent jaundice during pregnancy with details on clinical course No biopsies, no or scarce laboratory data	12	Table 15
IV	Recurrent jaundice during pregnancy number of gestations mentioned only	26	Table 20
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TABLE 11 Liver biopsies in recurrent intrahepatic cholestasis of pregnancy (Category I)

Year	Author	Author's case number	Number of pregnancy	Time of biopsy (days)*	Duration of jaundice at biopsy (weeks)
1956	Ljunggren	?	1	early pp	?
1958	Gros	1	8	late pp	
1959	Svanborg & Ohlsson	?	?	2 pp	6
				ap	?
				pp	?
1959	Dolle & Martini	11	4	p	11
1960	Pieragnoli et al	1	2	3 pp	2
1961	Belvedere & Finotti	1	2	5 ap	4
		2	2	ap	4
1962	Cahill	1	2	?	3-4
		2	5	?	?
1962	Dietel	1	2	7 pp	3-4
1962	Hausheer & Lauer	1	4	1 pp	11
		1	5	10 pp	1
1963	Beraud et al	1	2	pp	4
1963	King & Kerrins	1	3	3 pp	3
1963	Moore	1	6	4 pp	4
		2	3	early pp	?
		2	4	at p	11
1964	Ikonen	L A	2	6 pp	?
		S V	5	ap	<1
		A S	2	14 pp	?
		A P	5	21 pp	<1
1966	Haemmerli & Wyss	B	2	21 pp	3
		C	2	3 pp	1
		E	3	2 pp	2
		F	6	at p	2

* ap=before delivery, pp=after delivery at p=on day of delivery

enterological Associations, September 30th 1960, in Zurich) and was first employed as title in a report describing own cases by Orellana and Osorio in 1963. The term is also used in Sherlock's textbook.

Henceforward we will employ the

terms 'intrahepatic cholestasis of pregnancy and recurrent intrahepatic cholestasis of pregnancy' to designate a specific disease. The term 'recurrent jaundice during pregnancy' will be used as a descriptive one for the classification of diseases of different etiology.

Findings	Needle biopsy	Surgical biopsy taken during	Abdominal operations without liver biopsy
Marked cholestasis	+		
Minimal cholestasis	+		
Mild cholestasis	+	(Peritoneoscopy)	
Cholestasis irregular and focal	+		
Normal	+		
Normal		Caesarian section	
Mild cholestasis	+		
Minimal cholestasis	+		
Centroacinar bile pigment in liver cells	+		
Cholestasis	+		
Cholestasis	+		
Cholestasis	+		
Minimal cholestasis	+		
Normal	+		
Focal cholestasis	+		Exploratory laparotomy T-drain in common duct
Cholestasis	+		
Normal	+		Interval cholecystectomy
Minimal cholestasis	+		
Minimal cholestasis		Caesarian section	
Cholestasis	+		
Normal		Abdominal hysterotomy	
Normal	+		Caesarian section
Normal		Abdominal hysterectomy	
Centroacinar bile pigment in liver cells		Exploratory laparotomy	Surgical stenulation
Minimal cholestasis	+		
Mild cholestasis	+		
Minimal cholestasis		Abdominal hysterotomy	

Material for review of world literature

Whenever an attempt is made to delineate a newly emerging disease entity by reviewing all published case reports in the literature it appears wise to include in such a review only cases in which the diagnosis is beyond doubt. In

intrahepatic cholestasis of pregnancy no parameter singly or in combination with others is specific and none will provide an unquestionably correct diagnosis. However two features will allow to restrict any errors in diagnosis to an acceptable minimum.

TABLE 11 Liver biopsies in recurrent intrahepatic cholestasis of pregnancy (Category I)

Year	Author	Author's case number	Number of pregnancy	Time of biopsy (days)*	Duration of jaundice at biopsy (weeks)
1956	Ljunggren	1	1	early pp	2
1958	Gros	1	8	late pp	6
1959	Svanborg & Ohlsson	1	2	2 pp	2
				ap	2
				pp	2
1959	Dolle & Martini	11	4	p	5
1960	Pieragnoli et al	1	2	5 pp	2
1961	Belvedere & Finotti	1	2	3 ap	4
		2	2	ap	4
1962	Cahill	1	2	2	3-4
		2	5	2	2
1962	Dietel	1	2	7 pp	3-4
1962	Hausheer & Lauer	1	4	1 pp	6
		1	5	10 pp	1
1963	Beraud et al.	1	2	pp	4
1963	King & Kerrins	1	3	5 pp	3
1963	Moore	1	6	4 pp	4
		2	3	early pp	2
		2	4	at p	11
1964	Ilkonen	L.A.	2	6 pp	2
		S.V.	5	ap	<1
		A.S.	2	14 pp	2
		A.P.	5	21 pp	<1
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		C	2	3 pp	1
		E	3	2 pp	2
		F	6	at p	2

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Henceforward we will employ the

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Findings	Needle biopsy	Surgical biopsy taken during	Abdominal operations without liver biopsy
Marked cholestasis	+		
Minimal cholestasis	+		
Mild cholestasis	±	(Pentotomocopy)	
Cholestasis, irregular and focal	+		
Normal	+		
Normal		Caesarian section	
Mild cholestasis	+		
Minimal cholestasis	+		
Centroacinar bile pigment in liver cells	+		
Cholestasis	+		
Cholestasis	+		
Cholestasis	+		
Minimal cholestasis	+		
Normal	+		Exploratory laparotomy
Focal cholestasis	+		T-drain in common duct
Cholestasis	+		
Normal	+		Interval cholecystectomy
Minimal cholestasis	+		
Minimal cholestasis		Caesarian section	
Cholestasis	+		
Normal		Abdominal hysterotomy	
Normal	+		Caesarian section
Normal		Abdominal hysterectomy	
Centroacinar bile pigment in liver cells		Exploratory laparotomy	Surgical sterilisation
Minimal cholestasis	+		
Mild cholestasis	+		
Minimal cholestasis		Abdominal hysterotomy	

Material for review of world literature

Whenever an attempt is made to delineate a newly emerging disease entity by reviewing all published case reports in the literature it appears wise to include in such a review only cases in which the diagnosis is beyond doubt. In

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1962	Hausheer & Lauer	1	4	1 pp	6
		1	5	10 pp	1
1963	Beraud et al	1	2	pp	4
1963	King & Kerrins	1	3	5 pp	3
1963	Moore	1	6	4 pp	4
		2	3	early pp	?
		2	4	at p	11
1964	Ikonen	L.A.	2	6 pp	?
		SV	5	ap	<1
		A.S.	2	14 pp	?
		A.P.	5	21 pp	<1
1966	Haemmerli & Wyss	II	2	21 pp	3
		C	2	3 pp	1
		E	3	2 pp	2
		F	6	at p	2

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Henceforward we will employ the

terms intrahepatic cholestasis of pregnancy and 'recurrent intrahepatic cholestasis of pregnancy' to designate a specific disease. The term 'recurrent jaundice during pregnancy' will be used as a descriptive one for the classification of diseases of different etiology.

TABLE 12 Liver biopsies in non recurrent intrahepatic cholestasis of pregnancy (Category V II A)

Year	Author	Total cases	Histological diagnosis	
			Cholestasis	Normal
1947	Nixon et al. (case 14)	1	1	—
1953	Puyo (case 4)	1	1	—
1956	Ljunggren	6	5	1
1959	Svanborg & Ohlsson	4	3	1
1961	Katz et al.	4	3	1
1961	Van Woert & Kirsner	1	—	1
1963	Brown et al.	3	3	—
1963	Myhre	1	—	1
1963	Orellana & Osorio	8	7	1
1964	Ikonen	2	1	1
1964	Gros	2	2	—
1966	Haemmerli & Wyss (unpublished)	2	2	—
	Combined	33	28	7

The only truly abnormal finding is confined to the centroacinar area. Some bile canaliculi contain bile plugs. The canaliculi are of normal caliber or occasionally slightly dilated. The liver cells surrounding the centroacinar bile canaliculi contain bile pigment and may show an accumulation of fine basophilic granules. The portal tracts are not involved.

The histological picture is consistent with the diagnosis of intrahepatic cholestasis. In only one case (Ljunggren) is cholestasis described as marked. In 15 others bile stasis is mild or even minimal. In 2 instances only biliary pigment in the centroacinar liver cells and no capillary bile plugs were found. In 5 cases liver biopsy was described as normal. Three authors have stressed the fact, that cholestasis, if present is irregular and focal (Svanborg and Ohlsson Be-

raud et al. Haemmerli and Wyss). On different sections from the same biopsy bile plugs may be present in one slide and absent in the other (Haemmerli and Wyss). If cholestasis is not carefully looked for, it may be missed as often only a few bile thrombi are seen on one slide and bile pigment deposition in the liver cells is not impressive. This may explain 3 normal readings: the biopsies of which were taken before delivery in 1, during delivery in 1 and 4 days after delivery in a third case. The 2 other normal readings occurred in biopsies taken 14 and 21 days after delivery where cholestasis may well have disappeared.

In 2 cases 2 biopsies were taken during the same pregnancy (Ljunggren, Svanborg and Ohlsson). The cholestasis had regressed in both instances during the second biopsy taken after delivery. In another case with a biopsy taken 3

- 1 recurrence of the same syndrome (defined on page 36) during successive pregnancies
- 2 a liver biopsy compatible with intrahepatic cholestasis

These two criteria are met in 14 reports covering a total of 23 patients. In only 18 of these sufficient information is available on clinical course and laboratory data. These 18 patients underwent a total of 70 pregnancies, 4 of which terminated in an early abortion, 9 were uncomplicated, 11 were associated with pruritus only and 47 showed the full syndrome with pruritus and jaundice. These 47 pregnancies will form the basis for our review.

A combined total of 132 cases of recurrent jaundice during pregnancy has been published in the literature. For the purpose of this review the cases have been divided into 6 categories according to their documentation (see Table 10). Occasionally it has become necessary to allot different cases contained in a single report into different categories.

Category I will be used to discuss liver biopsy findings, categories I A and II to discuss laboratory data. Categories I A and II A will serve for the description of the clinical course. The 61 cases in categories III, IV and V provide insufficient data for a critical evaluation. The 28 cases in category VI do definitely not belong to the disorder called intrahepatic cholestasis of pregnancy and will be discussed under the section on differential diagnosis.

The 267 patients with the non recurrent form of (possible) intrahepatic cholestasis of pregnancy (category VII) are added to complete the review. They

will be discussed but briefly to point out some discrepancies with the confirmed cases or to point out some possible diagnostic pitfalls.

The reports covering category I and II cases originate from Sweden, Finland, England, Ireland, Germany, Switzerland, France, Italy, Poland, Canada, the United States of America and Chile. Additional countries contributing to category III—V cases are Holland, Austria, Roumania and Argentina and a category VII case is reported from Belgium.

Liver biopsies

In 25 pregnancies of 23 patients a total of 27 liver biopsies have been performed (category I, see Table 11). Of the 27 biopsies 20 were blind percutaneous needle biopsies, 1 consists of a needle biopsy taken during peritoneoscopy and 6 are surgical biopsies (Seydl's case with a normal biopsy 2 months after the disappearance of jaundice has been put into category II).

The findings are rather uniform. The liver architecture is intact. There is no liver cell damage. Neighbouring liver cells look alike. Minor deviations from the normal, consistent with those usually seen in uncomplicated pregnancy occur as an expression of an increased regenerative activity. They consist of Kupffer cell proliferation and mobilization, mild thickening of the framework, PAS positive granules in the Kupffer cells, some PAS containing macrophages in the portal spaces and of incipient ballooning of liver cells in the centroacinar areas.

normal biliary system at operation (Moore case 1)

The liver was considered to be of normal appearance in 10 instances and to be slightly enlarged in 1 patient (Ilonen, case A P). The spleen was found to be normal in all cases. During peritoneoscopy Gros described the liver as being of normal size, shape and consistency, its surface as smooth, glistening, of grey brown colour with a greenish tint. A close up view revealed a fine greenish stippling.

One case in category I had gallstones (Haemmerli and Wyss case F). They were discovered after the 6th pregnancy. No cholecystectomy was done. This woman had only a single questionable episode of biliary colic 11 years previously and none during the next 16 years. Three further patients of categories II—V had gallstones. The patient of Katz et al (category II B) underwent a cholecystectomy for cholelithiasis after her 3rd pregnancy. Her first pregnancy was asymptomatic, her 2nd to 5th complicated by pruritus only and her 6th to 8th were associated with pruritus and jaundice. Case O S of Ilonen (category IV) was cholecystectomized for gallstones after her 2nd pregnancy and was jaundiced again in her 3rd gestation. Another patient with gallstones is Cahill's case 4 (category V, no details).

In Moore's case 2 (category I A) the gallbladder was removed after her 6th pregnancy despite of a normal pre-operative cholecystogram and despite of normal findings at operation. Interestingly enough her next pregnancy was asymptomatic. Her first 3 pregnancies were however asymptomatic too. Non

icteric pregnancies following icteric ones have been observed in 3 instances without cholecystectomy and without evidence of gallbladder disease (King and Kerns, Ilonen case A S, Haemmerli and Wyss, case C).

These several observations make a causal relationship between gallstones and jaundice, or between cholecystectomy and absence of jaundice unlikely.

Radiological gallbladder examinations were performed in 8 of the surgically explored cases and in an additional 6 patients without surgical procedures in category I. Of the latter normal results were obtained in all (Cahill case 1, Pieragnoli et al, King and Kerns, Hausheer and Lauer, Ilonen case L A, Haemmerli and Wyss case C). Of the patients with normal cholecystography after delivery three had a previous radiological examination during the height of jaundice, all showing non filling of the gallbladder (Beraud et al, Hausheer and Lauer, Haemmerli and Wyss, case C). In category II A cholecystograms were performed in 3 cases with normal results (Jodkowski and Chojcka, McAllister and Waddell, Simmons).

A very interesting observation was made in the case of Beraud et al. In this woman jaundice set in in the second month of gestation and lasted until after delivery at term. At 4 1/2 months of gestation an exploratory laparotomy was performed. Despite normal macroscopic findings and a normal operative cholangiogram a T-tube was inserted into the common duct. Bile flow from this tube was normal in volume, colour and viscosity and the intensity of jaun-

weeks after delivery biliary pigment was found in the centroacinar liver cells, but bile canaliculi were normal. Apart from this rapid improvement after delivery, no correlation appears to exist between intensity of histological cholestasis and intensity of jaundice, duration of icterus before biopsy or time relation of biopsy to date of delivery.

It is remarkable how little impressive histological cholestasis in this disease is when compared with the clear-cut clinical and biochemical "obstructive pattern", especially considering the often violent pruritus of the patients.

A further 35 biopsies have been performed in the non recurrent form of intrahepatic cholestasis of pregnancy (category VII A, see Table 12). The histological findings were identical, with 28 biopsies showing cholestasis and 7 read as normal. The 3 cases of so-called "hepatitis" during pregnancy with normal liver biopsies reported by Ingerslev and Teilum in 1951 may represent 3 further examples.

The 3 non-recurrent cases biopsied by Brown et al. were examined by electron microscopy. The microvilli of the bile capillaries were swollen and occupied most of the lumen. Within the liver cells dilatation and vacuolization of the profiles of the endoplasmic reticulum was seen, with occasional complete desintegration of ergastoplasm. No controls in uncomplicated pregnancy were done and no recurrent case of intrahepatic cholestasis of pregnancy has yet been examined by electron microscopy. Furthermore two of the three cases were clinically atypical: case 1 had splenomegaly and case 2 no pruritus and a prodromal

phase of malaise, weakness and anorexia. For these reasons, the findings of Brown et al. cannot yet be accepted as representative for recurrent intrahepatic cholestasis of pregnancy.

Biopsies have been performed in 6 other instances of recurrent jaundice during pregnancy (Nixon et al. cases 16 and 19, Perreau and Rouchy, case II, Caroli et al. case 3, Lebon et al., Justin-Besançon et al.). The histological findings differed from those described above, but so did the clinical and biochemical findings. These cases do not represent intrahepatic cholestasis of pregnancy and will be discussed under the section on differential diagnosis.

Gross anatomical findings and radiological gallbladder examinations

No woman died of the disorder and no post mortem examinations have been performed.

Ten of the 23 patients in category I underwent 11 surgical abdominal interventions, in 7 of which liver biopsies were taken (see Table 11). The interventions consisted of 3 surgical interruptions of gestation in the 3rd or 4th month because of jaundice (Ikonen cases S V and A P, Haemmerli and Wyss, case F), 2 exploratory laparotomies because of jaundice (Beraud et al., Haemmerli and Wyss, case B), 1 peritoneoscopy because of jaundice (Gros), 3 Caesarian sections (Moore case 2, Dolle and Martini, Ikonen case A S), 1 surgical sterilisation on the day of spontaneous delivery (Haemmerli and Wyss, case B) and 1 cholecystectomy in the interval between 2 pregnancies with

TABLE 13 Clinical course in recurrent intrahepatic cholestasis of pregnancy Cases verified by liver biopsy (category 1A)

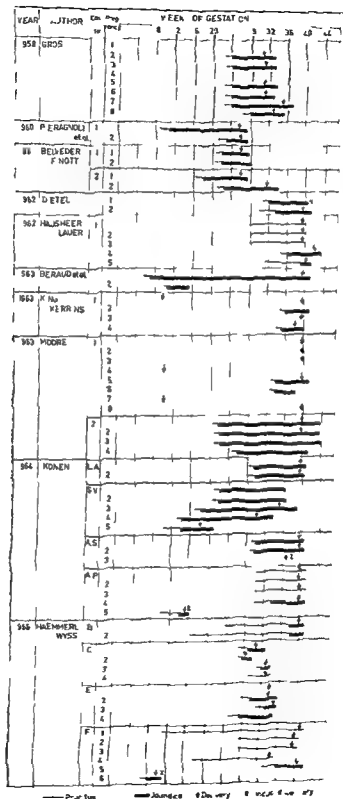


TABLE 14 Clinical course in recurrent intrahepatic cholestasis of pregnancy Cases without liver biopsy but with adequate laboratory data (category II A)

YEAR	AUTHOR	Country nancy	WEEKS OF GESTATION											
			4	8	12	16	20	24	28	32	36	40	44	
1955	SVANBERG	I	1											
			2											
			3											
		2	1											
			2											
			3											
		3	1											
			2											
		6	1											
			2											
1961	JODKOVSKI + CHOJECKA	1	1											
1961	PERREAU + ROUCHY	II	1											
			2											
			3											
		IV	1											
			2											
			3											
			4											
		IX	1											
			2											
			3											
			4											
			5											
		IX	1											
			2											
			3											
			4											
			5											
			6											
			7											
			8											
1962	McALLISTER VINDOEL	1	1											
1962	SEYDL	1	1											
		2	2											
1963	MOORE	3	1											
1963	SYMONS	2	1											
		1	1											
1966	HEMMERLE WYSS	A	1											
			2											
		D	1											
			2											

TABLE 13 Clinical course in pruritic recurrent jaundice during pregnancy Cases without adequate laboratory data Possibly examples of recurrent intrahepatic cholestasis of pregnancy (category III)

YEAR	AUTHOR	Case nr	Preg non- cyclic	WEEKS OF GESTATION										
				4	8	12	16	20	24	28	32	36	40	44
1881 1899	AHLFELD	1												
		2												
		3												
		4												
1896	BECKING	1												
		2												
		3												
		4												
		5												
1923 1904	BAUER VON DEN VELDEN	1												
		2												
		3												
		4												
1906	MAYER	1												
		2												
1924	BOREEL	1												
		2												
		3												
		4												
		5												
1929	MAGNANI	1												
		2												
		3												
1932	SCHWALM	1												
		2												
		3												
		4												
		5												
		6												
		7												
		8												
		9												
		10												
		11												
		12												
1940	ERHAGE	1												
		2												
		3												
		4												
		5												
		6												
		7												
		8												
1953 1961	PERRAUD ROUCHY	1												
1962	GABRIEL BERNARD	1												
		2												
		3												
1964	IRONEN	1												
		2												
		3												
		4												
		5												
	AM	1												
		2												
		3												

dice did not change during the long drainage period

Symptoms and signs

The disease has been observed in all age groups during the childbearing period. The clinical course in categories I A, II A and III is graphically summarized in Tables 13, 14 and 15 respectively.

Pruritus is the first symptom to occur and dominates the clinical picture throughout the whole course in this disorder. Pruritus is usually violent and involves the trunk or the extremities or both. Most authors note that the palmar and plantar surfaces of hands and feet may be involved. When pruritus lasts for more than two weeks excoriations (scratch marks) usually appear on the skin. In a rare case they may become secondarily infected and result in an impetiginous rash (Haemmerli and Wyss first pregnancy of case F). Pruritus usually leads to insomnia which renders the women tired and irritable. Pruritus precedes jaundice usually by 1—2 weeks but may begin up to 22 weeks earlier (Haemmerli and Wyss, second pregnancy of case B).

In some instances pruritus is the only clinical symptom of liver dysfunction and jaundice does not occur at all. In these cases pruritus may be of long duration (up to 30 weeks in first pregnancy of case F by Haemmerli and Wyss). Pruritus gravidarum without jaundice appears to be a *forme fruste* of intrahepatic cholestasis of pregnancy.

Intrahepatic cholestasis of pregnancy has been called jaundice of late pregnancy and it has been stated that jaun-

dice always begins during the last 4 months of gestation (Svanberg and Ohlsson). This applies to the majority of the cases but there are many exceptions. Mean and median onset of jaundice among 47 icteric pregnancies in category I A is in the 26th week of gestation or just before the beginning of the last trimester. The observed range of onset varies between the 7th and the 39th week of gestation with 2 pregnancies in the 7—8th week, 2 in the 11th, 3 in the 14th, 2 in the 18th, 14 in the 22nd to 24th, 1 in the 26th, 9 in the 28th to 30th, 7 in the 32nd to 34th, 4 in the 35th to 36th and 3 pregnancies in the 38th to 39th week of gestation.

In 7 cases of category I A jaundice was noted between the 7th and 14th week of gestation. In addition jaundice began in the third month of gestation during the second to fifth pregnancy in Cahill's case 2 (category I B). Of 37 icteric pregnancies in category II A onset of jaundice was in 5 between the 10th and 16th week, in 5 between the 20th and 24th week, in 8 between the 26th and 28th week, in 8 between the 30th and 32nd week, in 7 between the 33rd and 36th week and in 4 between the 37th and 40th week. Category III includes possibly other forms of jaundice besides intrahepatic cholestasis of pregnancy. Still it may be of interest to note that icterus began in all 12 pregnancies of Schwalm's case in the first month of gestation. Onset of jaundice in the 3rd month occurred in 3 pregnancies of Boerels and in 3 pregnancies of Magnani's case.

Of course statistical figures dating onset of jaundice are in a way fallacious.

TABLE 15 Clinical course in pruritic recurrent jaundice during pregnancy Cases without adequate laboratory data Possibly examples of recurrent intrahepatic cholestasis of pregnancy (category III)

YEAR	AUTHOR	Case No.	Preg. Mon	WEEKS OF GESTATION											
				4	8	12	16	20	24	28	32	36	40	44	
1881 1898	AHLFELD	1													
		2													
		3													
		4													
1895	BECKING	1													
		2													
		3													
		4													
		5													
1903 1904	BAUER VON DEN VELDEN	1													
		2													
		3													
		4													
1906	MAYER	1													
		2													
1924	BOREEL	1													
		2													
		3													
		4													
		5													
1929	MAGNANI	1													
		2													
		3													
1932	SCHWALM	1													
		2													
		3													
		4													
		5													
		6													
		7													
		8													
		9													
		10													
		11													
		12													
1930	VERHAGE	1													
		2													
		3													
		4													
		5													
		6													
		7													
		8													
1953 1961	FERRE - ROUCAY	1													
		2													
1957	GABRIEL BERNARDIN	1													
		2													
		3													
1964	MONEY	1													
		2													
		3													
		AM													

may be difficult to elicit. On the other hand, liver and spleen size were normal in all 10 cases with surgical abdominal explorations. Easy bruising or echymosis would be expected in cases with prolonged prothrombin time (usually occurring in cases with jaundice of long duration) but has never actually been observed in these patients.

In no case of categories I and II did onset of jaundice precede onset of pruritus and in no case did jaundice disappear before delivery. After delivery, whether spontaneous or induced, jaundice decreases rapidly and disappears usually within 1–2 weeks. In 46 instances in category I A jaundice disappeared within 1 week in 18, within 2 weeks in 21, within 3 weeks in 1 and within 4 weeks in 6 instances. The 6 instances with prolonged post-delivery jaundice are confined to 2 patients: Moore's case 2 and Ilonen's case S V. Moore's case 2 forms an exception in another respect: the intensity of jaundice increased during the first postpartum week. The same phenomenon occurred once in a case of category II A (Jodkowski and Chojecka, second pregnancy). Jaundice lasted for 6 weeks after delivery in 3 pregnancies of the non-verified case of Brauer and Von den Velden (category III).

Pruritus disappears before jaundice subsides. The only dissenting opinion is that of Svanborg and Ohlsson. All other authors agree that pruritus may disappear completely on the first postpartum day in some cases and decreases markedly in intensity immediately after delivery in all other instances to disappear completely before jaundice vanishes.

There is no evidence that intrahepatic cholestasis of pregnancy leaves any residual liver damage, but long term and careful follow up examinations have been few in number. Slightly elevated alkaline phosphatase levels have been observed 4 months and 1 year after an icteric pregnancy in 2 cases (Haemmerli and Wyss, cases II and F), but repeat examinations later on were normal. In one case bromsulfalein retention was 24% in 45 minutes 2 months after delivery (Beraud *et al.*) and in another 7% 1 year after delivery (Ilonen, case S V). Long term follow up examinations of 1 to 5 years with normal results were obtained in 10 cases (Thorling, cases 39 and 56; Svanborg and Ohlsson, 3 cases; Cahill, case 1; Haemmerli and Wyss, cases A, B, D and F). Pavel's case 1 (category IV) developed probable hepatitis 3 months after the last of 5 gestations with jaundice, but recovered rapidly and was well at the age of 60 years.

Laboratory data

Laboratory data of patients in category I A and category II A are summarized in Tables 16 and 17 respectively. The values given are the most abnormal ones recorded in the individual pregnancy for each single test. The several test results given for a single individual were not necessarily obtained on the same day. For example, peak bilirubin levels were usually observed before delivery, but peak alkaline phosphatase levels a few days after delivery. Furthermore, few patients have been serially examined and the maximal abnormal tests summarized in the tables may well be submaximal.

The patient herself will notice her jaundice often well after its true onset. On the other hand serum bilirubin elevation was noted very early before onset of clinical jaundice in at least 3 instances with onset of jaundice in the first trimester. These patients were carefully followed by their physicians because of the history of their previous pregnancies with jaundice (Ikonen cases S V and A P, Haemmerli and Wyss, case F).

Duration of jaundice from its observed onset until spontaneous delivery varied in 42 instances of category I A between 1 and 33 weeks, with a mean of 8.1 and a median of 6 weeks. Duration of 1 to 2 weeks occurred in 7 instances, of 3 to 4 weeks in 7, of 5 to 6 weeks in 8, of 7 to 8 weeks in 6, of 10 to 11 weeks in 4, of 12 to 13 weeks in 4, of 15 to 18 weeks in 5 and of 33 weeks in 1 instance.

In category II A duration of jaundice from its observed onset until spontaneous delivery varied in 33 instances between 1 and 24 weeks, with a mean of 7.7 and a median of 5 weeks. Duration of 1 to 2 weeks occurred in 9 instances, of 3 to 4 weeks in 6, of 5 weeks in 3, of 8 to 9 weeks in 6, of 12 to 16 weeks in 5 and of 17 to 24 weeks in 4 instances.

During jaundice or just preceding its onset the urine is described as dark by all patients. The stool colour is light in some instances, definitely normal in others, and not noticed by the patient or physician in most cases.

The general health of the patients is not impaired during the jaundiced stage. They feel well — apart from the irritation and lack of sleep resulting from itching — and continue without diffi-

culties their daily work, including heavy manual labor (farmer's wife). This is in marked contrast to the reduced general condition in viral hepatitis. In intra hepatic cholestasis of pregnancy there are no prodromal or general symptoms such as fever, nausea, vomiting, liver pains, arthralgias, anorexia — again in contrast to the clinical picture in viral hepatitis.

The 3 cases reported in the two Italian papers are the only ones to have associated symptoms apart from pruritus and jaundice. The first symptom during the first pregnancy in all 3 cases was severe pain in the right upper quadrant of the abdomen, radiating to the right shoulder in two cases. In the patient of Pieragnoli et al and in case 1 of Belvederi and Finotti this was associated with fever up to 38° centigrade. These symptoms are probably not related to intra hepatic cholestasis of pregnancy and did not recur in the two cases during the second icteric pregnancy. Case 2 of Belvederi and Finotti had some pains in the right upper abdominal quadrant during two pregnancies. A cholecystogram was normal in the case of Pieragnoli et al. The 2 cases of Belvederi and Finotti had the same gastrointestinal symptoms already before their first pregnancy.

No woman in categories I and II presented evidence of toxemia of pregnancy. Toxemia and pyelitis were noted in Ikonen's case O S (category IV).

Physical examination is unremarkable except for the presence of jaundice and scratch marks. The liver is rarely and the spleen never palpable. Examination of these patients is usually performed near term palpatory findings

Transaminases Wróblewski units		Prothrombin time s	Thymol turbidity Mayer-Lagan units	Zinc sulfate flocc Munkel units	Cephalin choles flocculation	Takats reaction	Serum iron g/100 ml	Bromsulphalein retention %	Galactose tolerance ⁺ test
SGOT	SGPT								
			2				68		
25		53			-			↑	
25		53	4		-			↑	
		42	4					↑	
AA	AA	100				+	109		
34		100	57		(+)				
JO			7		0			22	
		100	7					25	✓
		70	2		0				
920		89	1		0				
			3						
			2						
			✓						
			1						
200									
107	230	33					110		
15	100								
95									
76	160							18	
30	22	60			0	✓	50-205		✓
69	50		2.2	32	0	✓	50-100	10	N
19	53	70			0	✓	45-130		
52	111	100	4.9	7.9	0	✓	115-180		
238	175	50	4.5	4.7	0		130		
90	90	85			0	✓	20-190		
106	145				0	✓	40-140		
142	20		3.5		11		90-155		
183	226	30			0	✓	130-250	13	✓
10	197	82	4.4	6.4	0	✓	355		

* Conversion from King Armstrong units.

case VI and 8.4 mg per 100 ml (McAlister and Waddell). The direct reacting serum bilirubin fraction determined in 10 instances of category IA is responsible for most of the total serum bilirubin

elevation. Bilirubinuria was found in 17 of 20 instances. Bilirubinuria may be short lasting or intermittent, and can therefore easily be missed (Thorling Haemmerli and Wiss). Urobilinogen is

TABLE 16 Recurrent intrahepatic cholestasis of pregnancy
Cases with detailed laboratory data and liver biopsy (Category I A)

Year	Author	Number of cases and pregnancy	Urine			Serum Bilirubin mg/100 ml		Alkaline phosphatase Bodanik units	Cholesterol mg/100 ml
			Bilirubin	Urobilin	Urobilinogen	Total	Direct		
1958	Gros	1-8	+		↑	4.6	2.9	N	221
1960	Pieragnoli et al	1-2	+	+		6.8	5.0		400
1961	Belvederi & Finotti	1-2	+	+		6.8	5.0		400
		2-2	+	+		5.3	4.0		386
1961	Dietel	1-2	+	-		3.5		13+	280
1962	Hausheer & Lauer	1-4				4.5	1.9	22*	314
		1-5				3.2	1.3	15*	
1963	Beraud et al	1-1	+			5.5	4.1		213
		1-2	+			5.6	4.2	28	240
1963	Yang & Kerrins	1-2				3.5	2.2		
		1-4				3.7	3.0	6.8	
1963	Moore	1-6				2.1		28	
		2-4				3.5		26	
1964	Ilonen	LA-2	+			2.8			
		SV-5				1.2		7	235
		AS-2				1.5			515
		AS-3				0.5		4	400
		AP-4	+			3.5		6.8	
		AP-5	-			1.2		4.2	
1966	Haemmerli & Wyss	B-1	(+)	-	N	3.5		11.6	287
		B-2	-	-	N	3.6		21.2	590
		C-1	+	+	N	5.9		17.5	290
		C-2	+	-	↑	3.6		14.6	450
		C-3	+	+	^	3.6		14.4	420
		E-2	+	-	N	4.2		11.4	
		E-3	+	-	N	4.5		13.4	360
		E-4	(+)	-	N	4.5		11.8	300
		F-5	(+)	(+)	N	5.0		12.6	333
		F-6	↑	-	^	3.1		12.7	200

N=normal ↑=increased +=positive -=negative * Conversion from Bessey Lowry units

because examinations have not been performed during the peak abnormality

Serum bilirubin determinations have been obtained in 29 pregnancies of the 18 patients in category I A. Jaundice is

always of mild degree. The maximal recorded value was 6.8 mg per 100 ml. This value is surpassed three times in category II A with 7.8 (Haemmerli and Wyss, case A), 8.0 (Perreau and Rouchy

Transaminases Wróblewska units									
SCOT	SCOT	Prothrombin time %	Thymol turbidity McLagan units	Zinc sulfate flocc Kunkel units	Cephalin cholesterol flocculation	Takata reaction	Serum iron g/100 ml	Bromsulphalein retention %	Galactose tolerance test
			2				68	✓	
23		53			-			✓	
23		53	✓					✓	
		42	✓						
AA	AA	100				+	109		
34		100	57		(+)				
50			N		0			22	
		100	7					25	N
		70	2		0				
910		89	1		0				
			3						
			2						
			✓						
200			1						
107	230	33					110		
3	100								
93									
76	160							18	
20	22	60			0	✓	50-200		✓
69	50		22	32	0		50-100	10	✓
9	53	70			0	✓	45-130		
32	53	100	49	79	0	✓	115-180		
238	175	50	45	47	0		130		
90	90	85			0	✓	20-190		
106	145				0	✓	40-140		
142	220		35		0		90-155		
183	226	30			0	✓	130-250	13	✓
150	197	82	44	64	0	✓	325		

* Conversion from King Armstrong units.

case VI and 84 mg per 100 ml (McAlister and Waddell). The direct reacting serum bilirubin fraction determined in 10 instances of category IA is responsible for most of the total serum bilirubin

elevation. Bilirubinuria was found in 17 of 20 instances. Bilirubinuria may be short lasting or intermittent, and can therefore easily be missed (Thorling, Haemmerli and Wyss). Urobilinogen is

TABLE 17 Recurrent intrahepatic cholestasis of pregnancy
Cases with detailed laboratory data, but no liver biopsy (Category II)

Year	Author	Number of case and pregnancy	Urine			Serum Bilirubin mg/100ml		Alkaline phosphatase Bodansky units	Cholesterol mg/100 ml
			Bilirubin	Urobilin	Urobilinogen	Total	Direct		
1954	Svanborg	1-2				3.8		10 ⁺	
		1-3				2.6		7 ⁺	
		2-1				3.5		28 ⁺	
		2-3				5.1		16 ⁺	
		3-2				2.6		10 ⁺	
1955	Thorling	4-2				1.6		8 ⁺	
		39-3				2.1		6 ⁺	
		51-2				3.0		14 ⁺	
		56-1				2.3		14 ⁺	
1961	Jodkowski & Chojacka				N	6.5		11.8	235
1961	Katz et al					3.6	1.4	15	334
1961	Perreau & Rouchy	IV-3	+			2.6			342
		V-4	+			4.0			460
		VI-2	+			8.0			170
		VIII-5	+			2.5		9*	388
		IX-8	+			6.8			240
1962	McAllister & Waddell		+			8.4	3.0	6.6*	
1962	Seydl					3.1		15	
1963	Moore	3-1				2.6	1.9	8*	
		3-2				3.9		16*	
1963	Simmons	1-1				2.5		15*	
		1-2	+			3.0		19*	
1966	Haemmerli & Wyss	A-2				7.8		■	231
		D-1	(+)	+	^	3.6		20.7	213
		D-2	-	+	^	2.4			384
		D-3	-	-	N	1.3		22.0	370
		D-4	-	-	^	0.8		19.4	350

N=normal ^-increased +=positive -- negative * Conversion from King Armstrong units

present in normal or increased amounts, but is never completely absent from the urine. Urobilin was found in 6 of 14 instances.

Consistent with the clinical and histological feature of cholestasis there is as a rule an elevation of alkaline phosphatase

and cholesterol. One or the other or even both these tests may be normal, however in an occasional case.

Alkaline phosphatase, determined in 22 pregnancies of 14 patients in category I A was normal in 2 pregnancies between 4 and 7 Bodansky units in 4

Transaminases Wróblewski units											
ECOT	SCPT	1 rothrombim time %	1 hr. col turbidity MacLagan units	Zinc sulfate flocc Kunkel units	Cephrin cholest flocculation	Takata's reaction	Serum iron, /100 ml	Bromsulphalein retention %	Galactose tolerance test		
			5			/					
			2			/					
			1								
			1			/					
			3			N					
			1			/					
		115	1			+					
		89	N	N	0						
		96	3								
		^									
57	94	100	N			N		16			
		100									
		77									
80	48		8		2+			16			
	↑	80									
					0						
			1								
			2								
			3								
			N	2							
			2	2							
		37				N			N		
91	77				+	N	40-160				
					+	N	55-160	23	N		
66	18	100	3	51	(+)	N	100-160	23			
■	27				0	N	100-200				

* Conversion from Buch-Buch units.

pregnancies and between 11 and 28 Bodansky units in 16 gestations. The findings in category II A correspond in general to the ones in category I A. As most figures given for category II A had to be converted into Bodansky units from other units, no data will be tabu-

lated. Serum cholesterol, determined in 13 pregnancies of category I A, was below 250 mg per 100 ml in 3 and between 250 and 590 mg per 100 ml in 10 gestations. In 12 pregnancies of category II A, serum cholesterol varied between 170 and 460 mg per 100 ml.

The elevation of serum cholesterol is seldom more pronounced than that seen in uncomplicated pregnancies (Ikonen), whereas serum alkaline phosphatase elevation is — when present — usually much more marked than the usual elevation observed towards term in uncomplicated gestations (Thorling). There is no correlation in the single case between elevation of serum bilirubin, alkaline phosphatase or cholesterol.

Serum electrophoresis, performed in 15 pregnancies of 5 patients reported by Haemmerli and Wyss, fits also into the pattern of biochemical cholestasis. There is a decrease in serum albumin, a slight increase of alpha-1 and a moderate increase of alpha-2 globulins, a usually pronounced increase in beta globulins and normal or slightly decreased gamma globulins. In every case the beta globulin fraction is higher than the gamma-globulin fraction. The electrophoretic changes represent an exaggeration of those seen in uncomplicated pregnancy. Total proteins are somewhat decreased as in normal gestation.

The serum turbidity and flocculation tests are normal as a rule, with few, but only borderline exceptions (Perreau and Rouchy, case VIII).

Prothrombin time is normal or only slightly prolonged in the majority of cases. It may be prolonged to critical levels whenever jaundice is of long duration. This is the only test that shows such a correlation. Of 17 pregnancies in category I A prothrombin time was below 60 % in 7 instances, 3 of which being around 30 %. Prolongation of prothrombin time is entirely due to a deficiency in the Vitamin K dependent

coagulation factors II, VII and X (Haemmerli and Wyss) and is readily corrected by the application of Vitamin K.

Serum glutamic oxalacetic transaminase may be normal or increased. In 21 pregnancies of 12 patients in category I A it was normal in 4, between 50 and 100 units in 9, between 100 and 240 units in 7 patients and 920 units in the case reported by King and Kerrins. Serum glutamic pyruvic transaminase, determined in 14 pregnancies of 7 patients, was normal in 1, between 50 and 100 units in 6 and between 100 and 230 units in 7 gestations. It was not determined in the case with the exceptionally high SGOT. Besides the high SGOT found in the case of King and Kerrins, 2 other recurrent cases of Ikonen (not specified which ones) had similar high levels: SGOT 716 and SGPT 875 in one case and SGOT 450 and SGPT 565 in the other. SGPT was higher than SGOT in 9 of 16 cases with both determinations (category I A and Ikonen's cases). Five cases in category II with determination of the transaminases show mild to moderate elevations.

The 3 cases with very high transaminases are astonishing. As they fit in all other regards into the general picture of intrahepatic cholestasis of pregnancy, and as one of these cases has been biopsied, they are tentatively accepted in this review. For practical diagnostic purposes, however, it appears wise for the present to regard a level of 250 units as the upper limit usually seen in this disease.

Serum 1 phospho-fructidolase was elevated to 84 units and serum sorbit

dehydrogenase up to 76 units in case E of Haemmerli and Wyss. In serial determinations during two pregnancies of this patient both enzymes paralleled the behaviour of the serum transaminases closely.

Bromsulfalein retention is always increased during jaundice. The recorded values in 8 cases of category I A and 4 cases in category II varied between 10% and 25% after 45 minutes. A special modification using a single intravenous injection of 800 mg bromsulfalein was employed in the three Italian cases of category I A. This test revealed a decreased hepatic uptake of bromsulfalein and an increased cholestatic index in all.

In contrast to the increased bromsulfalein retention, urinary galactose excretion after a 40 g oral loading dose is always normal (4 tests in category I A and 2 in II A in 4 of 6 tests simultaneous bromsulfalein test).

There is no evidence of hemolysis in this disorder. No erythrocyte survival studies have been performed and a mild hemolysis as a contributory phenomenon is therefore not excluded. Hemoglobin is usually normal for pregnancy. The lowest recorded hemoglobin levels are 63% (Hausheer and Lauer), 66% (Haemmerli and Wyss, second pregnancy of case B) and 67% (Jodkowski and Chojacka). Reticulocyte counts are not increased. Urobilinogenuria cannot be used as a criterium for hemolysis in the presence of disturbed liver function. Serum iron levels behave erratically (Ikonen, Haemmerli and Wyss) with both abnormally low and abnormally high levels (up to 355 microgm per 100 ml) in the same pregnancy. Nearly all in-

creased levels were found before delivery, decreased levels both before and after. There is no correlation between serum iron and hemoglobin. There is also no correlation between serum iron and serum transaminases such as occurs in viral hepatitis (Haemmerli and Wyss).

Total leucocyte and differential counts are normal or show a leucocytosis and/or shift to the left as in uncomplicated pregnancies. Non protein nitrogen is never elevated.

Laboratory data before and after delivery

Long and serial pre delivery laboratory examinations have been performed only in the 6 cases reported by Haemmerli and Wyss. These periods were of 14, 15, 22, 24, 62 and 123 days duration. It is probable but not conclusively proved that an elevation of the serum transaminases first indicates the onset of the full syndrome. However, bilirubin elevation lags but a few days behind. Serial observations in cases with a long preicteric pruritic phase are necessary to settle this question. Serum alkaline phosphatase and serum cholesterol are usually elevated before the serum bilirubin or the transaminases. The significance of this elevation is difficult to evaluate as both these parameters are increased in the last trimester of most normal pregnancies.

In cases with jaundice of short duration serum bilirubin usually increases towards delivery. In cases with jaundice of long duration serum bilirubin rapidly reaches a plateau. It may remain steady or fluctuate and even decrease slightly.

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TABLE 18 Recurrent intrahepatic cholestasis of pregnancy
Cases with liver biopsies but no details on clinical course (category I B)

YEAR	AUTHOR	CASE NR	PREGNANCIES
1956	LJUNGGREN		■ ■
1959	SVANBORG+OHLSSON		■ ■
1959	DOLLE+MARTINI		■ ■ ■ ■
1962	CAHILL	1	■ ■
		2	□ ■ ■ ■ ■

TABLE 19 Recurrent intrahepatic cholestasis of pregnancy
Cases with laboratory data but no details on clinical course (category II B)

YEAR	AUTHOR	CASE NR	PREGNANCIES
1955	THORLING	39	□ □ ■ ■
		51	■ ■
		56	■ ■
1961	KATZ et al		□ ■ ■ ■ ■ ■ ■ ■ ■
1961	PERREAU+ROUCHY	VI	■ ■ ■

Legend for Tables 18 and 19 see Table 20

43 pregnancies with jaundice and spontaneous delivery in the 18 cases of category I A there were 23 premature deliveries and 20 deliveries at or near term. However this kind of statistics is treacherous in a way because 22 premature deliveries occurred in 7 women and 19 deliveries at term in 10 women. In these cases every delivery of the single individual was either at term or premature (see Table 13). In just one case is a premature delivery combined with a delivery at term (Heraud et al).

These general conclusions are also evident in category II A. Premature delivery correlates neither with time of onset of jaundice in relation to stage of pregnancy nor with the intensity or the duration of jaundice. Furthermore premature delivery appears to be independent of intrahepatic cholestasis of pregnancy itself. Case E of Haemmerli and Wyss delivered prematurely in 4 pregnancies, the first of which was uncomplicated. Similarly case C of Haemmerli and Wyss delivered prematurely

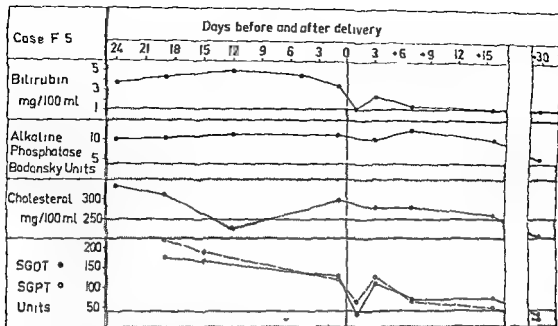


Fig 1 Example of laboratory data in recurrent intrahepatic cholestasis of pregnancy (case F fifth pregnancy, Haemmerli and Wyss 1966)

toward delivery (Haemmerli and Wyss, case A)

After delivery serum bilirubin and serum transaminases begin to decrease immediately and are, together with urinary bile pigments, the first tests to become normal again. Serum alkaline phosphatase may continue to increase for 4 to 10 days after delivery (Haemmerli and Wyss) and is the last test to become normal. Mild elevations may persist for up to 2 months after delivery.

A curious phenomenon was observed by Haemmerli and Wyss in 2 cases. Immediately after delivery there was a transient "dip" or return to normal of serum bilirubin and serum transaminases, only to reach pre-delivery levels again within 24 hours. A gradual improvement occurred thereafter (see Fig 1). It is not clear whether this phenomenon is a rule or an exception. Because of its short lasting transient nature it will

easily be missed if serial laboratory determinations in 8 to 10 hour intervals are not performed during the first day after delivery.

Obstetrical course, incidence of premature deliveries and child survival

No obstetrical complications occurred during delivery in category I A. There is a single mild complication in category II A: patient D reported by Haemmerli and Wyss had a blood loss of 750 ml during her first delivery. Her prothrombin time was 37 % of normal. The child's prothrombin time was 0 % and it died despite immediate Vitamin K therapy from massive intracranial hemorrhage. The bleeding in the mother stopped spontaneously and did not necessitate blood transfusions.

The incidence of premature deliveries is high in the combined series. Among

TABLE 18 Recurrent intrahepatic cholestasis of pregnancy
Cases with liver biopsies but no details on clinical course (category I B)

YEAR	AUTHOR	CASE NR	PREGNANCIES
1956	LJUNGGREN		1 2
1959	SVANBORG-OHLSSON		1 2
1959	DOLLE-MARTINI		1 2 3 4
1962	CAHILL	1	1 2
		2	1 2 3 4 5

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		56	1 2
1961	KATZ et al		1 2 3 4 5 6 7 8
1961	PERREAU-ROUCHY	VI	1 2 3

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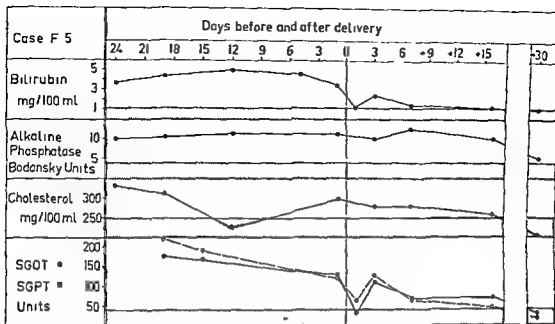


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1959	DOLLE + MARTINI		■ ■ ■ ■
1962	CAHILL	1	■ ■
		2	□ ■ ■ ■ ■

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		56	■ ■
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1961	PERREAU + ROUCHY	VI	■ ■ ■

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TABLE 20 Recurrent jaundice during pregnancy
Cases reported with few details (category IV)

YEAR	AUTHOR	CASE NR	PREGNANCIES
1902	MULLERHEIM		□ □ □
1910	NASON		□ □ A □ □ □ □ □ □ □ □
1910	ROLLESTON		■ ■ ■ ■
1924	BOREEL	mother of case	□ □ □ □ □ □ □
1935	PEL		□ □ □ □ □
1953	PUYO	2	■ ■ ■
1954	DOWIE		□ □
1955	MEYER	1	□ □
		2	□ □
1957	PAVEL et al	1	■ ■ ■ ■ ■ ■ A
		2	■ ■ ■ ■ ■ ■
1958	WILKEN	2	□ □
1959	SVANBORG + OHLSSON		■ ■ ■
			■ ■
			■ ■
		sister	■ ■
1961	PERREAU + ROUCHY	VII	□ A □
		sister of case VI	□ □ □ □ □ □
1961	ORELLANA et al		■ ■
			■ ■ ■
1961	SHEEHAN		■ ■ ■
1963	ORELLANA + OSORIO		■ ■ ■ ■
			□ □ □ □ ■ ■ ■
1964	IKONEN	OS	■ ■
		LV	■ ■
		MS	□ ■ ■

- uncomplicated pregnancy
■ pregnancy with pruritus
■ pregnancy with pruritus and jaundice

- pregnancy with jaundice
pruritus not mentioned not negated
A abortion

in 4 pregnancies the last of which was complicated by pruritus without jaundice

Thus premature delivery appears to be a feature of the single individual. The overall high incidence remains still to be explained. Perhaps whatever mechanism causes intrahepatic cholestasis of pregnancy may independently also cause premature delivery, with no direct causal relationship between jaundice and premature delivery.

Of 23 children born prematurely 10 died shortly after birth. Of the 20 children born after the 36th week 6 died during delivery mainly from intrauterine asphyxia. The already mentioned death of an infant with a prothrombin time of 0.5% from intracranial bleeding and a similar incident in a non-recurrent case of Thorlings are probably unrelated to the jaundiced mother's coagulation deficiency because extremely low prothrombin times are encountered not infrequently in newborns from healthy mothers.

No baby was jaundiced in categories I and II. Several jaundiced babies were observed in 3 non-verified cases of category IV (Nason, Rolleston, Pavel et al case 1). Erythroblastosis foetalis is not excluded in these cases, 2 of which were reported in 1910. There were 2 congenital malformations in category III, both occurring in the same mother (case I P. Ilonen), the first child died with ileal atresia 6 weeks after birth and the second was stillborn with a ventricular septal defect. Jaundice began during the 5th month in the first and during the 8th month in the second gestation so that a causal relation

TABLE 21 Recurrent jaundice during pregnancy. Cases without any details reported (Category V)

Year	Author	No of cases
1872	Hoffman	1
1903	Kehrer	4
1923	Fppinger	1
1927	Holmer	1
1937	Wewalka	3
1961	Mecroff	1
1962	Cahill (cases 3 and 4)	2
1962	Friedberg	4
1963	Orellana and Osorio	2
1964	Ilonen	4
Total		23

ship to the malformations is very unlikely.

Clinical course of successive pregnancies in the individual patient

The pregnancies of categories I A, II A and III are summarized in Tables 13, 14 and 15 respectively, the pregnancies of categories I B, II B and IV in Tables 18, 19 and 20. Category V cases without details are mentioned in Table 21.

A glance at Tables 13 and 14 will rapidly show that the clinical course in this disease is by no means uniform. About the only existing rule is that jaundice occurs some time before delivery and disappears rapidly thereafter, whether delivery is spontaneous or induced.

It has been stated by Stanborg and Ohlsson that the disease runs a similar course in the individual patient in repeated pregnancies. This generalization is true only to a certain extent and is

TABLE 20 Accidents jaundice during pregnancy
Cases reported with few details (category IV)

YEAR	AUTHOR	CASE NR	PREGNANCIES
1902	MULLERHEIM		□ □ □
1910	NASON		□ □ A □ □ □ □ □ □ □ □
1910	ROLLESTON		■ ■ ■ ■
1924	BOREEL	mother of case	□ □ □ □ □ □ □
1935	PEL		□ □ □ □ □
1953	PUYO	2	■ ■ ■
1954	DOWIE		□ □
1955	MEYER	1	□ □
		2	□ □
1957	PAVEL et al	1	■ ■ ■ ■ ■ ■
		2	■ ■ ■ ■ ■ ■ A
1958	WILKEN	2	□ □
1959	SVANBORG + OHLSSON	sister	■ ■ ■
			■ ■
			■ ■
			■ ■
1961	PERREAU + ROUCHY	VII	□ A □
		sister of case VI	■ ■ ■ ■ ■ ■
1961	ORELLANA et al		■ ■
			■ ■ ■
1961	SHEEHAN		■ ■ ■
1963	ORELLANA + OSORIO		■ ■ ■ ■
			□ □ □ □ □ □ □
1964	IKONEN	OS	■ ■
		LV	■ ■
		MS	□ ■ ■

- uncomplicated pregnancy
 ■ pregnancy with pruritus
 ■ pregnancy with pruritus and jaundice

- pregnancy with jaundice
 pruritus not mentioned not negated
 A abortion

serum bilirubin levels of 3.6, 2.4, 1.3 and 0.8 mg per 100 ml in 4 successive pregnancies

In category V there are 2 remarkable cases. In the patient of Dowse the interval between the first and second pregnancy with jaundice was 12 years. The case of Nason had jaundice only in the 4th to 8th of her 11 pregnancies. In the 7th and 8th pregnancy jaundice disappeared about 10 days before delivery, so that the possibility of diagnosis other than intrahepatic cholestasis of pregnancy exists.

No definite statement as to the frequency of recurrence in successive pregnancies can be made because non recurrent cases have been excluded from this survey. Thorling reported 10 multiparas with gestation after an icteric pregnancy. Three had again pruritus and jaundice, two pruritus only and 5 were asymptomatic during the next pregnancy.

Treatment

Intrahepatic cholestasis of pregnancy is a benign disorder with full recovery after delivery. One hesitates even to call it liver disease. From the standpoint of therapeutic consequences it might as well be regarded as just a disordered function of little importance. Neither bed rest nor a dietary regimen are necessary and the patient may continue her normal daily life. Whenever jaundice is of longer duration (more than 2 weeks) the prothrombin time should be checked regularly. If this is not practicable Vitamin K can be given prophylactically.

Pruritus, the most annoying symptom, does not respond to antihistaminics

(Gros, Haemmerli and Wyss). Itching is easily abolished with cholestyramine, an ion exchange bile acid sequestrant, in the dose of 10 gm per day. When the drug is stopped pruritus recurs within 1 to 2 days (Haemmerli and Wyss). Cholestyramine was also successfully employed in the non recurrent case 3 of Brown et al. and in a case of pruritus gravidarum with slightly elevated alkaline phosphatase, cholesterol and transaminases but no jaundice (Haemmerli and Wyss, unpublished observation).

Intrahepatic cholestasis of pregnancy is definitely not an indication for an induced termination of pregnancy. An induced termination of pregnancy may lead to the loss of the child. It will certainly cure the mother's jaundice but this will be cured anyhow after spontaneous delivery. As pruritus can now be easily controlled with cholestyramine there is no symptom which could demand a shortening of the jaundiced period. Today induced terminations of pregnancy are usually performed by physicians who are not familiar with the disorder and mistake it for a serious liver disease.

Antecedent or underlying hepato biliary or gastrointestinal disease

The past history of patients with intrahepatic cholestasis of pregnancy is usually non revealing except for the usual childhood diseases. Case B of Haemmerli and Wyss (category I A) had probable viral hepatitis at the age of 8 years. Nonspecific upper gastrointestinal disturbances before the first pregnancies were noted for 1 and 9 years respectively in the 2 cases of Belvedere and Finotti.

even contradicted by the 4 cases reported by Svanborg in detail 5 years earlier (category II A). An apparent similarity exists in the course of 8 of 18 cases in category I A. However, among these 8 cases only the one of Belveden and Finotti is reported with exact details of all pregnancies. In the other 7 reports (Gros, Dietel, Hausheer and Lauer, 4 cases of Ikonen) the descriptions of all except the last pregnancy of the cases are lumped together (for example: "Jaundice usually appeared in the 24th week and lasted until 2 weeks after delivery"), and therefore we assume that the "similarity of the course" is more fictitious than real. If a history on previous pregnancies is obtained during the last pregnancy only, not too much weight should be put upon a woman's recollection. Wherever detailed charts for each pregnancy were available for retrospective review (as in the series of Haemmerli and Wyss) similarity is less striking.

Jaundice does not necessarily occur during every pregnancy of the single individual. Jaundice in every pregnancy was present in only 9 of 18 cases in category I A. Seven of these 9 cases underwent 2 pregnancies only and may therefore not be representative for the full spectrum of possibilities in this disease.

Jaundice when present is not necessarily of the same intensity during successive pregnancies. Jaundice of equal intensity was noticed in the same 9 of 18 cases mentioned above: 7 patients with 2 pregnancies, 1 with 4 (Moore case 2) and one with 5 pregnancies (Ikonen, case S V).

The other 9 patients in category I A

show a varying symptomatology during successive gestations. It may be significant, that all these cases underwent 3 or more gestations (mean 5.2, maximum 8).

In the patient reported by King and Kerns only the second and the fourth pregnancy were associated with pruritus and jaundice, whereas the first and third were uncomplicated. In case 1 of Moore only 2 of 6 fullterm pregnancies were symptomatic, the first three and the last one being uncomplicated.

In 5 cases the syndrome appears to progress in intensity during successive pregnancies. In 3 patients the first 3 or 4 pregnancies were complicated by pruritus only, the following two showing the full syndrome with jaundice (Hausheer and Lauer, Ikonen case A, P, Haemmerli and Wyss case F). In the other 2 cases the first pregnancy was asymptomatic, with the full syndrome in the following gestations (Gros, Haemmerli and Wyss case E). In addition Cahill's case 2 (category I B) conforms to this pattern, but no details are given.

In 2 cases a definite improvement is noted in successive pregnancies with the last one being completely asymptomatic (case A S by Ikonen case C by Haemmerli and Wyss).

Among the 15 cases in category II A (Table 12) jaundice was about equal in successive pregnancies in 12 patients, 8 of which with 2, 3 with 3 and 1 with 4 pregnancies. A progression in intensity is shown in the cases VIII and IX by Perreau and Rouchy with the first several pregnancies asymptomatic or pruritic only. An instructive example of decreasing intensity is provided by case D of Haemmerli and Wyss with peak

serum bilirubin levels of 3.6, 2.4, 1.3 and 0.8 mg per 100 ml in 4 successive pregnancies

In category V there are 2 remarkable cases. In the patient of Dowie the interval between the first and second pregnancy with jaundice was 12 years. The case of Nason had jaundice only in the 4th to 8th of her 11 pregnancies. In the 7th and 8th pregnancy jaundice disappeared about 10 days before delivery, so that the possibility of diagnosis other than intrahepatic cholestasis of pregnancy exists.

No definite statement as to the frequency of recurrence in successive pregnancies can be made because non recurrent cases have been excluded from this survey. Thorling reported 10 multiparas with gestation after an icteric pregnancy. Three had again pruritus and jaundice, two pruritus only and 5 were asymptomatic during the next pregnancy.

Treatment

Intrahepatic cholestasis of pregnancy is a benign disorder with full recovery after delivery. One hesitates even to call it liver disease. From the standpoint of therapeutic consequences it might as well be regarded as just a disordered function of little importance. Neither bed rest nor a dietary regimen are necessary and the patient may continue her normal daily life. Whenever jaundice is of longer duration (more than 2 weeks) the prothrombin time should be checked regularly. If this is not practicable Vitamin K can be given prophylactically.

Pruritus, the most annoying symptom, does not respond to antihistaminics

(Gros, Haemmerli and Wyss). Itching is easily abolished with cholestyramine, an ion exchange bile acid sequestrant, in the dose of 10 gm per day. When the drug is stopped pruritus recurs within 1 to 2 days (Haemmerli and Wyss). Cholestyramine was also successfully employed in the non recurrent case 3 of Brown et al and in a case of pruritus gravidarum with slightly elevated alkaline phosphatase, cholesterol and transaminases but no jaundice (Haemmerli and Wyss, unpublished observation).

Intrahepatic cholestasis of pregnancy is definitely not an indication for an induced termination of pregnancy. An induced termination of pregnancy may lead to the loss of the child. It will certainly cure the mother's jaundice but this will be cured anyhow after spontaneous delivery. As pruritus can now be easily controlled with cholestyramine there is no symptom which could demand a shortening of the jaundiced period. Today induced terminations of pregnancy are usually performed by physicians who are not familiar with the disorder and mistake it for a serious liver disease.

Antecedent or underlying hepato biliary or gastrointestinal disease

The past history of patients with intrahepatic cholestasis of pregnancy is usually non revealing except for the usual childhood diseases. Case III of Haemmerli and Wyss (category I A) had probable viral hepatitis at the age of 8 years. Nonspecific upper gastrointestinal disturbances before the first pregnancies were noted for 1 and 9 years respectively in the 2 cases of Belvederi and Finotti.

These symptoms recurred during their pregnancies (category I A). Ulcerative colitis of about 1 year's duration was diagnosed in the middle of the 12 year interval between the 2 pregnancies with jaundice of Dowic's case (category IV).

The 4 patients with cholelithiasis have already been mentioned in the paragraph on radiological gallbladder examinations.

Familial occurrence of intrahepatic cholestasis of pregnancy

Some patients with recurrent intrahepatic cholestasis of pregnancy have close relatives with a history of recurrent jaundice during pregnancy. None of these relatives has been examined in detail.

Jaundice during pregnancy occurred in 2 sisters of one of Cahill's cases (category I B or V, not stated which case). Case VI of Perreau and Rouchy (category II A) had a sister with jaundice during 6 successive pregnancies, each leading to premature delivery. Pruritus and jaundice were observed during one of several pregnancies in the mother of the case reported by both Brauer and by von den Velden (category III). The mother of Boreel's case (category III) was jaundiced during 7 pregnancies and the patient was the only surviving child, all others dying from prematurity. Two pregnancies with jaundice occurred in the older sister of a patient reported by Svanborg and Ohlsson (category IV).

The mother of case S V reported by Ikonen (category I A) had pruritus without jaundice during the 4th, 5th, 10th and 11th of her 13 pregnancies.

Pruritus with jaundice occurred once in a maternal cousin of the same case.

The two sisters with recurrent jaundice during pregnancy reported by both Benedict and Lovrich probably represent another disorder as both had hepatomegaly and one a marked splenomegaly in addition. An older sister and the mother of these two cases had uncomplicated gestations.

In Mayer's case (category III) the mother died from liver carcinoma and the father and one sister suffered from "chronic liver disease".

An inherited predisposition to intrahepatic cholestasis of pregnancy can neither be accepted nor be excluded from these observations.

Non recurrent intrahepatic cholestasis of pregnancy

A total of 267 cases with a possible diagnosis of non recurrent intrahepatic cholestasis of pregnancy have been published in the literature. The laboratory data are summarized in Table 22. In 55 cases liver biopsies were performed (see Table 12). Associated cholelithiasis thought to be unrelated to jaundice was found in 17 of 267 patients.

Many of these cases are incompletely documented and many may belong to quite different diagnostic categories. In 15 patients jaundice was associated with severe pyelonephritis (Van Woert and Kirsner Laurijssens and Demeulenaere Comerford, case 4; Thorling case 42; Ikonen 11 cases) and in 3 cases jaundice was associated with pyelitis and hemolysis (Thorling cases 40, 41 and 43). Icterus of pregnancy was present in

case 10 of Comerford and in 27 of Ilonens cases. Two cases of Thorling had marked hypertension (number 64 and 67). Ten patients of Ilonen had anemia. Nine cases had no pruritus (Thorling 3 cases, Svanborg and Ohlsson 1 case, Brown et al case 2). Six cases listed are definitely not examples of intrahepatic cholestasis of pregnancy. In 4 of Thorling's cases jaundice disappeared well before delivery. Mähtes case had no pruritus, severe hypertension, marked hepatomegaly, an elevated serum amylase and a normal liver biopsy. Brown et al's case 1 had splenomegaly.

On the other hand 8 cases published as viral hepatitis in the original reports have been included in Table 22 on the basis of their clinical and biochemical data (Nixon et al case 14, Barry and O'Dwyer case 1, Comerford cases 1, 3, 5, 6, 7 and 9).

In Thorling's excellent monography on 72 patients with jaundice during pregnancy he writes: "Purely on the basis of the clinical signs and symptoms in the individual cases the diagnosis of viral hepatitis appears to be possible in practically all the patients. He selected from this group 35 cases on two criteria: jaundice appeared in late pregnancy and the thymol turbidity test was negative. While the majority of this group may truly represent intrahepatic cholestasis of pregnancy, many will not. Wewalka pointed out that the thymol turbidity test may not be reliable during pregnancy. In his series thymol turbidity was positive in 82% of patients with hepatitis outside of pregnancy but in only 33.5% of 58 cases with serum hepatitis (treatment for syphilis) during pregnancy.

The data in Table 22 are given for what they are worth. Just a few details will be pointed out.

It is remarkable that Orellana and Osorio collected their 59 cases within 2 years in a single hospital. Serum bilirubin levels are below 6.8 mg per 100 ml in all cases with one exception (case 2 of Brown et al without pruritus, 8.7 mg per 100 ml). Alkaline phosphatase, cholesterol, prothrombin time, turbidity and flocculation reactions correspond to those in verified cases of recurrent intrahepatic cholestasis of pregnancy. The one high bromsulfalein retention of 50% is from Brown et al's case 1 with splenomegaly. The biopsied case 2 of Gros had a 39% retention, whereas all other bromsulfalein retention tests performed are between 14 and 28% as in the verified cases. It will be remembered that in the verified category I A and II A cases the serum transaminases were below 250 units with three exceptions (King and Kerrins, Ilonen 2 cases). High values were also found in the non recurrent case 3 of Brown et al (SGOT 670, SGPT 220).

It must be concluded from this brief survey that the non recurrent form of intrahepatic cholestasis of pregnancy is extremely difficult to diagnose with absolute certainty. The disease can easily be simulated by viral hepatitis with a benign course occurring late in pregnancy or by drug induced intrahepatic cholestasis except that both these diseases have typical prodromal symptoms. For a diagnosis of non recurrent intrahepatic cholestasis of pregnancy an absence of 'hepatitis-like' prodromi and the rigid clinical, biochemical and histo-

TABLE 22 Non recurrent intrahepatic cholestasis of pregnancy (267 cases)
Diagnosis mostly not verified (Category VII)

Year	Author	Number of cases	Number of biopsies	Number of cholecystograms		Urine bilirubin	Serum bilirubin mg/100 ml	
				Total	Stones		Total	Direct
1947	Nixon et al (case 14)	1	1				35	14
1953	Puyo (cases 4 & 6)	2	1			+	16-50	
1954	Svanborg	3		2	2		21-51	
1955	Barry & O'Dwyer	1					20	
1955	Thorling	35		17	2		<5.8	
1956	Ljunggren	51	6	34	5		<6.5	
1959	Svanborg & Ohlsson	18	4	18	4		20-68	
1961	Katz et al.	7	4				<5.4	<3.0
1961	Orellana et al	12					21-68	0.5-2.4
1961	Van Woert & Karsner	1	1			+	2.2	1.5
1962	Comerford	6					16-50	
1962	Laurijssens & Demeulenaere	1					46	
1963	Brown et al	3	3				22-87	13-28
1963	Moore	6						
1963	Muller & Felsch	20						
1963	Myhre	1	1	1			15	21
1963	Orellana & Osorio	59	11	21		+	13-48	0.3-3.1
1964	Ilkonen	35	2	32	4	+	10-50	A
1964	Gros	1	2	1		+	20-35	
1966	Haemmerli & Wyss (unpublished)	3	2			+	24-58	

N=normal A=increased + =positive ° Conversion from Buch-Buch units * Conversion from

logical criteria emerging from the review of the verified recurrent cases (category I A) must be demanded

Pruritus is the prominent and probably compulsory symptom but the presence of itching alone does not make the diagnosis of intrahepatic cholestasis of pregnancy. Pruritus appears to occur more frequently in all liver diseases during pregnancy than in liver disease outside of pregnancy. Thorling found pruritus in 17 of 23 cases of viral hepatitis during pregnancy (74 %) and believes that the pregnant state in some way favours the manifestation of this symptom in the presence of hepatitis. Martini et al observed itching in 12 of 17 cases of hepatitis during pregnancy. Pruritus occurs also in hyperemesis gravidarum with jaundice and may be present in this disease without jaundice (Thorling). In two cases of acute fatty

liver disease during pregnancy, pruritus was observed in 17 of 23 cases of viral hepatitis during pregnancy (74 %) and believes that the pregnant state in some way favours the manifestation of this symptom in the presence of hepatitis. Martini et al observed itching in 12 of 17 cases of hepatitis during pregnancy. Pruritus occurs also in hyperemesis gravidarum with jaundice and may be present in this disease without jaundice (Thorling). In two cases of acute fatty

Alkaline phosphatase Bodansky units	Cholesterol mg/100 ml	Transaminases Wróblewski units		Prothrombin time °	Thymol turbidity	Different flocculation tests	Bromsulphalein retention °
		SGOT	SGPT				
8-30°	630				✓	✓	
14				↑	✓	✓	
6-28°				3 ↑	✓	✓	
~20°				4 50-70	✓		
				8 < 50			
<26°				7 ^	✓	✓	
<16+	<310	<76	<148	>50	✓	✓	14-24
12-36	210-331			83-100	✓	✓	
15	230			100	✓	✓	
6 21+					✓		
15		40	25		✓		
13 14+	<249	55-640	62-220	✓	✓		28-50
		30-260	17-297				
11+		112	80	N	N	✓	
9-36	29>300			13↑	N	✓	
5-20		2>300	2>300	20-100	4 ^		
5 8		26-68	57-75	100	✓	✓	39
11-18	240-330	45-246	39-340	✓	✓	✓	

King Armstrong units * Conversion from Belsey Lowrey units.

metamorphosis of pregnancy marked pruritus was noted (Ober and Lecompte case 1 CPS case) Itching was also a feature in the two cases of jaundice with hemoglobinuria during pregnancy (Schaeffer Meinhold)

Pruritus gravidarum

Itching has been defined as an unpleasant cutaneous sensation which

provokes the desire to scratch (Thorling) or as one of the seemingly mild symptoms which seems humorous to observers but which may be desperately serious to the patient' (Kasdon) Itching is the most frequent cutaneous disturbance during pregnancy and occurs in a generalized or localized form the latter as abdominal vulvar or anal pruritus Both forms usually occur during the second half of gestation

Abdominal pruritus has been noted by Kasdon in 7% of 42 women in the first trimester, in 20.9% of 110 women during the second trimester and in 18.3% of 213 women during the third trimester. The etiology is unknown.

The term pruritus gravidarum should be reserved for the generalized form. No statistics exist as to its frequency, but the syndrome is well known to all obstetricians. Circumstantial evidence favors the concept that pruritus gravidarum is a *forme fruste* of intrahepatic cholestasis of pregnancy. First of all, many cases of recurrent intrahepatic cholestasis of pregnancy will present with recurrent pruritus without jaundice during their first few gestations. Well documented examples have been published by Katz et al., Perreau and Rouchy, case IX, Hausheer and Lauer, Orellana and Osorio, Ikonen, case A, P and Haemmerli and Wyss, case F. Secondly, in all cases of intrahepatic cholestasis of pregnancy jaundice is preceded and accompanied by pruritus. Thirdly, pruritus gravidarum has its onset in the second or third trimester and disappears with or shortly after delivery, i.e. its clinical course in relation to stage of gestation is the same as the course of intrahepatic cholestasis of pregnancy. Lastly, cases of pruritus gravidarum show some disturbed liver functions which lie between those observed as physiological derangements during normal pregnancy and those seen in intrahepatic cholestasis of pregnancy.

Arfvedson, 1953 and 1956 compared 100 pregnancies with pruritus gravidarum to 100 without. Serum bilirubin in those with pruritus was between 1 and

2 mg per 100 ml in 42% and above 2 mg per 100 ml in 23%, compared to only 6% of determinations greater than 1 mg per 100 ml in normal pregnancy. Pathological bile components were found in 51% of those with pruritus gravidarum and in 5% of normal pregnancies. In 1956 Arfvedson and von Studnitz examined 42 women with pruritus gravidarum and 46 without during the last trimester. In those with pruritus there was a significant increase in total serum lipids, serum cholesterol, phospholipids and beta lipoproteins, whereas alpha lipoproteins were decreased. On serum electrophoresis there was a decrease in albumin and an increase in alpha 2 and beta 2 globulins in those with pruritus. Borglin found a SGOT elevation to 93 units in 1 out of 7 cases. Serum ornithyl carbamyl transferase was increased in 24 of 30 patients with pruritus (80%) compared to only 15% pathological results in normal pregnancies (Reichard et al.).

Few detailed case studies have been performed. In Thorling's 3 cases (number A, B and C) the serum bilirubin, thymol turbidity and cephalin flocculation were normal and the alkaline phosphatase was increased up to 35 Buch and Buch units. Laboratory data in the 2 cases reported by Topp and Charles were urine bilirubin positive, serum bilirubin 1.5 mg per 100 ml, alkaline phosphatase 35 King-Armstrong units and bromsulfalein retention 30% in 30 minutes in case 1, urine bilirubin positive, serum bilirubin 1.0 mg per 100 ml, alkaline phosphatase 62 King-Armstrong units, cholesterol 295 mg per 100 ml, bromsulfalein retention 35% at 30

minutes, transaminases, prothrombin time and flocculation tests normal in case 2. In an unpublished observation of Haemmerli and Wyss (wife of the second author) the following laboratory data were obtained: no abnormal urine bile components, total bilirubin 0.9 mg per 100 ml, direct reacting bilirubin 0.3 mg per 100 ml, alkaline phosphatase 112 Bodansky units, serum cholesterol 330 mg per 100 ml, SGOT 69 units, SGPT 63 units, 1 phosphofructaldolase 4.3 units (normal up to 28 units), normal results for prothrombin time, serum iron and thymol turbidity.

Therapeutically testosterone effectively controls itching but increases serum bilirubin levels (Arfvedson and von Studnitz). In the case of Haemmerli and Wyss cholestyramine in moderate doses (6 gm per day) brought complete relief but itching recurred upon each attempted cessation of the drug. Cholestyramine was also successfully used in a case reported by Brown et al.

Pruritus gravidarum, once it occurs, has a marked tendency to recur in successive pregnancies (Thorling, Topp and Charles case 2) and may lead to intrahepatic cholestasis of pregnancy in a later gestation (Bjerregaard, Perreau and Rouchy case III).

3: Differential diagnosis of recurrent intrahepatic jaundice during pregnancy

The 28 cases in category VI are all examples of recurrent jaundice during pregnancy in which intrahepatic cholestasis of pregnancy can be excluded with near certainty on the basis of the pub-

lished data. It is likely that some cases in categories III, IV and V would also have to be classified under a different heading if more data were provided for a critical evaluation of the original reports.

In 13 of the 28 cases the authors' original diagnosis has been accepted in this review. In 11 cases a tentative reclassification was made (Perreau and Rouchy case II, Puyo cases Le II and B S, Nixon et al case 16, Tylecote, Lantuejoul and Chambrud, Ezes and Bourdon, Lebon et al) and 5 cases could not be classified at all (Benedict, Lovrich, Vigues, Boquien et al, Justin-Besançon et al). Sometimes our reclassification will appear arbitrary and occasionally different opinions as to the etiology of a particular case may be possible even where a liver biopsy has been performed. For these reasons the case reports are recorded in some detail in this chapter.

Recurrent jaundice during pregnancy due to recurrent viral hepatitis or due to exacerbation of chronic anicteric hepatitis

Until recent years most French authors were convinced that recurrent jaundice during pregnancy is in all instances caused by viral hepatitis (Caroli et al). In no case such an etiology is proved. In three cases in which liver biopsies were performed it may be possible.

The patient of Albano and Albano contracted viral hepatitis during her first pregnancy. After typical prodromal symptoms she developed jaundice in the 7th month with a large tender liver, serum bilirubin up to 27.6 mg per 100

ml (direct reacting bilirubin 20.7 mg per 100 ml), both serum transaminases up to 1,100 units, positive zinc sulfate and thymol turbidity tests, normal alkaline phosphatase and bilirubinuria. She delivered several weeks later and was discharged with mild subicterus 2 weeks after delivery. In the interval she had some dyspeptic symptoms but no jaundice. Seven months later she was pregnant again. During the first month fever, dyspepsia, liver pains and subicterus set in. In the 5th month the serum bilirubin was 1.9 and the direct reacting bilirubin 1.0 mg per 100 ml. The urine was positive for bilirubin and urobilinogen, and there was an increase in gamma-globulins. On electrophoresis alkaline phosphatase, the transaminases and all flocculation and turbidity reactions were normal. By the 7th month bilirubin had risen to 10.3 mg per 100 ml, SGOT to 300 units, SGPT to 400 units and the flocculation reactions were now positive. Liver biopsy showed an intact lobular architecture and massive round cell infiltration of the periportal spaces. On bed rest and medical treatment including steroids she improved, delivered at term and has since been anicteric.

It is possible that jaundice during the second pregnancy in this case is due to either a true recurrence of the initial viral hepatitis, dormant after the first pregnancy, or that it represents a clinical manifestation of an anicteric chronic hepatitis under the stress of pregnancy.

Case II reported by Perreau and Rouchy had pruritus followed by jaundice in the 3rd month of her first two pregnancies, both times with premature

deliveries in the 6th month and subsidence of jaundice afterwards. During the second pregnancy bilirubinuria was present, serum bilirubin was 19.8 mg per 100 ml (direct reacting bilirubin 14.9 mg per 100 ml), alkaline phosphatase was 86 Bodansky units, prothrombin time was normal and the flocculation reactions were negative. A liver biopsy 9 days after delivery showed dislocated hepatic cell plates, enlarged liver cells with clarification of the cytoplasm, hyperplasia of the Kupffer cells and a mild increase in connective tissue. This was interpreted as "diffuse hepatitis." Jaundice disappeared 3 weeks after delivery and she was asymptomatic during the next 4 years. She then became pregnant for the third time and developed pruritus followed by jaundice in the 5th month. Serum bilirubin was 15.8 mg per 100 ml, bilirubinuria was present, and the cephalin flocculation was 1+. She delivered at 7 1/2 months and jaundice disappeared one week later. Six months after delivery she had a large and firm spleen, a serum bilirubin of 1.5 mg per 100 ml and no excretion of contrast material during radiological gallbladder examination. At present she has the full picture of liver cirrhosis verified by liver biopsy (personal communication from Dr. Perreau).

This case clearly developed toward liver cirrhosis while presenting with jaundice during 3 successive pregnancies. Again, chronic hepatitis is most likely the underlying primary disease.

Another possible example of chronic underlying liver disease after hepatitis with exacerbation during pregnancy is a case published by Puvion (Minc 1963).

observation of Cachera also published by Caroli et al and by Lacomme) This woman had viral hepatitis of mild degree at age 22 At age 23 she became icteric during the 4th month of her first pregnancy and had dark urine and light stools She delivered at 8 1/2 months and jaundice disappeared one month later During her second pregnancy she became jaundiced in the 4th month delivered in the 6th and was cured one month afterwards Six months later she had an enlarged firm liver and spleen a serum bilirubin of 3.2 mg per 100 ml and a positive cephalin flocculation Liver biopsy showed hyperplasia of the architecture of the liver cell plates During her third pregnancy she was again jaundiced before her delivery at term

Recurrent jaundice of pregnancy due to incipient primary biliary cirrhosis

A case published by Tylicote in 1914 may well represent an example of primary biliary cirrhosis This woman underwent 11 pregnancies during the age of 18 to 32 years In each pregnancy there was an insidious onset of jaundice with bilirubinuria during the 3rd month of gestation She usually delivered prematurely during the 7th or 8th month of gestation and jaundice did not clear until 3 months after delivery Since the last pregnancy she remained permanently jaundiced her liver was markedly enlarged and massive xanthomatosis appeared With the rapid succession of her multiple pregnancies and the long duration of jaundice after delivery it is possible that this woman was more or less

permanently jaundiced already before the onset of her xanthomatosis

No definite diagnosis can be made in this case because eight pregnancies would be very unusual in primary biliary cirrhosis Ahrens et al reported 17 females with this disease, 10 of which had an uncomplicated pregnancy prior to the clinical manifestation of their cirrhosis Three were pregnant during their illness In two of these bilirubin levels were not followed at that time while in one there was a sharp drop of serum bilirubin and serum lipids during pregnancy, to rise again after a spontaneous abortion in the 4th month

Recurrent jaundice during pregnancy due to posthepatic hyperbilirubinemia

Posthepatic hyperbilirubinemia thoroughly discussed by Kalk on the basis of 163 cases is a syndrome indistinguishable from the familial Gilbert's syndrome except for the presence of viral hepatitis in the past history It is characterized by a mild elevation of the indirect reacting serum bilirubin fraction with a normal direct reacting bilirubin and normal results in all other tests used to evaluate liver function

Diemel has published a case with posthepatic hyperbilirubinemia during 2 pregnancies (case E. Fr.) Two years after a first normal pregnancy this woman had typical viral hepatitis During the next two pregnancies 2 and 4 years after her hepatitis respectively she became mildly jaundiced during the last trimester with an elevation of the indirect serum bilirubin to between 2.7 and 3.5 mg per 100 ml Hemolysis was excluded and all other liver tests were

ml (direct reacting bilirubin 20.7 mg per 100 ml), both serum transaminases up to 1,100 units, positive zinc sulfate and thymol turbidity tests, normal alkaline phosphatase and bilirubinuria. She delivered several weeks later and was discharged with mild subicterus 2 weeks after delivery. In the interval she had some dyspeptic symptoms but no jaundice. Seven months later she was pregnant again. During the first month fever, dyspepsia, liver pains and subicterus set in. In the 5th month the serum bilirubin was 1.9 and the direct reacting bilirubin 1.0 mg per 100 ml. The urine was positive for bilirubin and urobilinogen, and there was an increase in gamma-globulins on electrophoresis. Alkaline phosphatase, the transaminases and all flocculation and turbidity reactions were normal. By the 7th month bilirubin had risen to 10.3 mg per 100 ml, SGOT to 300 units, SGPT to 400 units and the flocculation reactions were now positive. Liver biopsy showed an intact lobular architecture and massive round cell infiltration of the periportal spaces. On bed rest and medical treatment including steroids she improved, delivered at term and has since been aicteric.

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Recurrent jaundice during pregnancy due to posthepatic hyperbilirubinemia

Posthepatic hyperbilirubinemia, thoroughly discussed by Kalk on the basis of 163 cases is a syndrome indistinguishable from the familial Gilbert's syndrome except for the presence of viral hepatitis in the past history It is characterized by a mild elevation of the indirect reacting serum bilirubin fraction with a normal direct reacting bilirubin and normal results in all other tests used to evaluate liver function

Dieterl has published a case with posthepatic hyperbilirubinemia during 2 pregnancies (case E Fr) Two years after a first normal pregnancy this woman had typical viral hepatitis During the next two pregnancies 2 and 4 years after her hepatitis respectively she became mildly jaundiced during the last trimester with an elevation of the indirect serum bilirubin to between 2.7 and 3.5 mg per 100 ml Hemolysis was excluded and all other liver tests were

normal jaundice disappeared within one week after delivery.

A second case of Dietel's represents posthepatic hyperbilirubinemia during one pregnancy only. This woman contracted viral hepatitis one year after her first normal pregnancy. Two years after the hepatitis she became pregnant for the second time and developed mild jaundice during the last trimester with elevation of the indirect serum bilirubin fraction to between 2.5 and 3.5 mg per 100 ml. Liver biopsy in this case was normal and jaundice cleared rapidly after delivery. Martini et al mention three similar cases without giving any details.

Recurrent jaundice during pregnancy due to gallstones in the common bile duct

It has already been mentioned that jaundice due to common duct stones is extremely rare during pregnancy. It is not astonishing therefore, that no documented cases of recurrent jaundice during pregnancy due to this condition exist. Two cases may possibly fall into this category. Rissmann described in 1910 a patient with several episodes of biliary colic before her first pregnancy. One week before term during her first pregnancy she had a similar attack followed by jaundice which disappeared after delivery. Jaundice with colicky pains in the right upper abdominal quadrant occurred again two weeks before delivery during her second pregnancy. Another example may be case 1 (Mme B S reported by Fayo and again by Caroli et al). This woman was jaundiced during the 6th month of her first pregnancy.

Jaundice disappeared after delivery in the 7th month. The patient was plagued with intermittent colicky pains in the gallbladder area ever since. During the next pregnancy she was jaundiced from the 7th month until after her delivery at term. Cholelithiasis was found on radiological examination and a cholecystectomy was performed some months later. During the third pregnancy she had again an episode of jaundice lasting 4 weeks during the 4th month of gestation but this time it cleared well before delivery.

Recurrent jaundice during pregnancy due to familial non hemolytic jaundice

Four cases of this syndrome have been reported in the literature. In all hemolysis has been excluded with a reasonable degree of certitude, but in none an exact diagnosis could be established (such as Gilbert's syndrome, the Rotor syndrome or the Dubin—Johnson syndrome).

Paschalis reports a case with constitutional hyperbilirubinemia and jaundice during 12 successive gestations. A case of *Cholemie familiale* published by Chabrol: a pupil of Gilbert had subicterus and an enlarged spleen when not pregnant and extremely intensive jaundice with epistaxis and melena during 3 successive gestations each time subsiding after spontaneous abortion. Two patients with chronic mild jaundice and a history of the same disorder in their fathers and in one instance in a twin sister are reported by Nonen. Case E. K. had jaundice with a serum bilirubin of 5.8 mg per 100 ml in her otherwise uncomplicated first pregnancy. The next

two pregnancies terminated in spontaneous abortions. The chronic subicterus deepened again during the 4th gestation, the patient developed some epigastric pain and the liver margin became palpable and tender. There was no bilirubinuria and the thymol turbidity was negative. A bromsulfalein clearance was interpreted as pointing to the Dubin-Johnson syndrome. Liver biopsy was refused and a cholecystogram normal. Case H. F. with chronic subicterus had first an abortion in the 3rd month. During the second pregnancy the serum bilirubin was 2.6 mg per 100 ml and no bilirubin was present in the urine. She aborted in the 5th month. In the third pregnancy serum bilirubin was 2.0 mg per 100 ml, alkaline phosphatase 4.3 Bessey-Lowry units and SGOT 39 units. Urine bilirubin was negative. Premature delivery took place in the 7th month. One year later the patient became febrile and jaundiced. Radiological examination revealed gall stones.

Recurrent jaundice during pregnancy due to hemolysis

Two examples of hemolysis with jaundice due to unknown cause are reported in the literature. The first seven pregnancies in the case of Bromberg et al. were uncomplicated. During the 8th gestation headache, dizziness, anorexia and jaundice were noted in the 7th month. Hemoglobin was 6.1 mg per 100 ml, reticulocyte counts markedly elevated and total serum bilirubin 2.2 mg per 100 ml with a negative direct Van den Bergh reaction. Urine bilirubin was absent and urobilinogen increased. The woman de-

livered at term and recovered rapidly thereafter. During the next four years no acute hemolytic crisis occurred, but her hemoglobin remained around 7.5 to 9 gm per 100 ml, reticulocyte counts remained elevated and the spleen became enlarged. She became pregnant for the 9th time and suffered from an acute hemolytic crisis in the 25th week with a drop in hemoglobin to 4.5 gm per 100 ml and a rise in serum bilirubin to 4.6 mg per 100 ml. After induced abortion she recovered rapidly. Radiological gall-bladder examination was normal.

The case of Zachariae had a normal first pregnancy followed by one with an abortion. She was anemic during her third gestation (hemoglobin 31 %). Towards the end of her 4th pregnancy the hemoglobin dropped to 37 % and jaundice with a serum bilirubin of 5.0 mg per 100 ml was present until delivery 3 weeks later. Splenomegaly was noted. During the next 2 years she had once a hemolytic episode without jaundice while suffering from thrombophlebitis. During the fifth pregnancy hemolysis with jaundice occurred in the third month and was cured by an induced abortion.

A third case of recurrent jaundice during pregnancy is due to congenital spherocytosis (Rimbsch and Beickert). The patient's father, the father's brother and mother had chronic mild jaundice while the patient herself was never jaundiced outside her pregnancies. During 4 consecutive gestations she developed hemolytic crisis with jaundice, the hemoglobin dropping each time to about 30 %. Each time hemolysis disappeared within 2 weeks after delivery. The spleen

normal Jaundice disappeared within one week after delivery

A second case of Dietel's represents posthepatic hyperbilirubinemia during one pregnancy only. This woman contracted viral hepatitis one year after her first normal pregnancy. Two years after the hepatitis she became pregnant for the second time and developed mild jaundice during the last trimester with elevation of the indirect serum bilirubin fraction to between 2.5 and 3.5 mg per 100 ml. Liver biopsy in this case was normal and jaundice cleared rapidly after delivery. Martini et al mention three similar cases without giving any details.

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Paschalis reports a case with 'constitutional hyperbilirubinemia' and jaundice during 12 successive gestations. A case of 'Cholemie familiale', published by Chabrol, a pupil of Gilbert, had subicterus and an enlarged spleen when not pregnant and "extremely intensive jaundice" with epistaxis and melena during 3 successive gestations, each time subsiding after spontaneous abortion. Two patients with chronic mild jaundice and a history of the same disorder in their fathers and in one instance in a twin sister are reported by Ilkonen. Case E. K. had jaundice with a serum bilirubin of 3.8 mg per 100 ml in her otherwise uncomplicated first pregnancy. The next

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The case of Zachariae had a normal first pregnancy, followed by one with an abortion. She was anemic during her third gestation (hemoglobin 51 %). Towards the end of her 4th pregnancy the hemoglobin dropped to 37 % and jaundice with a serum bilirubin of 5.0 mg per 100 ml was present until delivery 3 weeks later. Splenomegaly was noted. During the next 2 years she had once a hemolytic episode without jaundice while suffering from thrombophlebitis. During the fifth pregnancy hemolysis with jaundice occurred in the third month and was cured by an induced abortion.

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was enlarged and morphological blood examination was consistent with spherocytosis

A fourth case, reported by Schneider and Frahm, has a mixed etiology. This woman had anemia during her 3rd and 4th pregnancy, and hemolytic anemia with mild jaundice during her 5th and 6th pregnancy. Investigation revealed a large spleen and a megaloblastic bone marrow. After the cure of her pernicious anemia with Vitamin B₁₂ the red cell morphology was typical for congenital spherocytosis, which subsequently was discovered to exist also in 3 of her siblings.

Recurrent jaundice with hemoglobinuria during pregnancy

In 1902 both Schieffer and Brauer reported the case of an Italian woman with marked pruritus and mild jaundice in the second half of 6 successive pregnancies except during the third which was terminated early by a spontaneous abortion. General symptoms were mild and the jaundice cleared rapidly after delivery. The dark urine contained bilirubin, urobilin and in addition hemoglobin. A similar non-recurrent case was observed by Meinhold in 1903 in a primipara, with marked pruritus and hemoglobinuria, but without jaundice and without bile constituents in the urine. Both patients inhabited areas where malaria was common; both had no fever and both had negative tests for malaria.

No further case of pregnancy with hemoglobinuria has been reported since. It is therefore unlikely that such an entity exists in reality.

Recurrent jaundice during pregnancy due to severe pyelonephritis

The only acceptable case of recurrent jaundice during pregnancy due to pyelonephritis in the literature is the one published by Fruhinsholz in 1929. During the 4th month of her first pregnancy this woman developed fever, chills, pains in the right flank, anorexia and vomiting, followed by oliguria. In the 5th month, while fever continued, frank jaundice was observed with a large and tender liver. Massive pyuria continued. Purpura developed on the extremities. An induced abortion was performed and the woman recovered rapidly. During the 7th month of the second pregnancy there was again pyuria, dysuria, pollakisuria and finally oliguria, shortly followed by jaundice with a large tender liver. This time there was no temperature elevation. The woman delivered 2 weeks later and jaundice disappeared rapidly.

Since the advent of sulfonamides and antibiotics no similar cases have been observed.

Recurrent jaundice during pregnancy due to hyperemesis gravidarum

A well documented case of this disorder is presented by Thorling (case 33). This woman had hyperemesis during her first pregnancy without jaundice and developed pruritus during the last month of gestation. During the second pregnancy she had again marked hyperemesis starting in the 11th week, followed shortly by itching, dark urine and manifest jaundice. There was urobilinuria, no bilirubinuria, an increase in serum bilirubin and alkaline phosphatase and a normal thymol turbidity. Upon hospitalisation

the symptoms subsided rapidly and she delivered without further complications at term. During the next pregnancy she had again hyperemesis at the time last noted during her whole gestation with intermittent episodes of dark urine and jaundice. Pruritus developed during the last two months. Upon delivery she became immediately symptom-free.

Another possible example is the case reported by Lantuejoul and Chambraud. This woman had hepatitis at 12 years of age. During her first two pregnancies she suffered from marked hyperemesis without jaundice and delivered prematurely both times. The sequence of events was the same in her third pregnancy but this time dark urine and jaundice were noted before delivery in the third month. During the 4th pregnancy hyperemesis started in the 6th month and jaundice in the 7th. Bilirubin and urobilin were present in the urine, serum bilirubin rose to 5.7 mg per 100 ml, the thymol turbidity and the cephalin flocculation were positive. After delivery a few weeks later she rapidly recovered.

There are no reported cases in which detailed laboratory data and in none has a liver biopsy been performed (see also page 3). Jaundice due to hyperemesis is clearly distinct from intrahepatic cholestasis of pregnancy by the presence of vomiting and by its cure well before delivery.

Recurrent jaundice during pregnancy with different etiology of jaundice in successive pregnancies

Three case reports will illustrate the diagnostic difficulties encountered in some cases of recurrent jaundice during

pregnancy. In these jaundice of different origin occurred in successive pregnancies or jaundice was of mixed etiology.

Case 16 reported by Nixon et al. had an abortion during the 6th week of her first gestation. In the 20th week of her second pregnancy she developed anorexia, nausea and vomiting, then dark urine and light stools followed by frank jaundice in the 24th week. Liver and spleen were enlarged, serum bilirubin rose to 18.2 mg per 100 ml and liver biopsy revealed typical acute viral hepatitis. She delivered in the 30th week and jaundice cleared only within 2-3 months. Five months later a liver biopsy was normal. One and a half years later she was again pregnant. During the 5th month she became icteric. The total serum bilirubin was 3.4 mg per 100 ml with normal amounts of the direct reacting fraction and hemoglobin dropped to 45%. In the 7th month total bilirubin was 1.4 mg per 100 ml. While this patient had proved viral hepatitis during the second pregnancy it appears probable that she had hemolytic jaundice during the third.

Less clear is a case reported by Eves and Bourdon. This woman was jaundiced from the 5th month until term in her first two pregnancies and jaundice cleared rapidly after delivery. Jaundice again became apparent in the 6th month of her 3rd pregnancy. Examinations at that time revealed a total serum bilirubin of 9.4 mg per 100 ml (direct reacting 6.4 mg per 100 ml), a low serum cholesterol, a prolonged prothrombin time and an increase of gamma globulins to 33% on electrophoresis.

She had a hematocrit of 36 % and a reticulocyte count of 6 %. Here, hemolysis appears to be an additional factor to whatever was the primary disease, which could have been chronic hepatitis or cirrhosis of the liver.

A similar case has been observed by Lebon et al. During the first two pregnancies this woman was jaundiced from the 7th month until shortly after her deliveries in the 8th month. During her third pregnancy she developed asthenia and pruritus in the 5th month, followed by intense arthralgias, back and chest pains, and then by jaundice. The liver was enlarged and tender, her general health markedly impaired, with a weight loss of 13 kg. Bilirubinuria was present, total serum bilirubin was 2.4 mg per 100 ml, alkaline phosphatase 15.8 Bodansky units, the flocculation reactions normal, serum cholesterol low, and liver biopsy showed a granular degeneration of the liver cells, an increase in Kupffer cells and an increase in reticulum. This was interpreted as "periportal hepatitis." Her red cell count was 2,700,000 per cu mm and reticulocytes were 3.4 %, so that hemolysis appears superimposed to what was — from the history — viral hepatitis during her 3rd pregnancy. The episodes of jaundice during her first two pregnancies are not explained. Hypothetically it is possible that a woman has recurrent intrahepatic cholestasis during two pregnancies and then viral hepatitis during the third.

Recurrent jaundice during pregnancy due to unclassified causes

Five cases of recurrent jaundice during pregnancy cannot be classified at all.

Two sisters with recurrent jaundice during pregnancy were reported by both Benedict and Lovrich. Pruritus and jaundice occurred in case 1 during 4, in case 2 during 2 gestations. In both cases onset of jaundice was in the first trimester. During the fourth pregnancy of case 1 the liver and the spleen were markedly enlarged and hard; during the second pregnancy of case 2 the liver was enlarged and the spleen was not palpable but enlarged to percussion. Cirrhosis of the liver or a familial hemolytic disorder (urine constantly negative for bilirubin) are not excluded in these cases.

The case described by Vignes in 1937 with jaundice during the 7th to 10th pregnancies has been considered to represent hemolytic jaundice by the author. The patient had tachycardia, dyspnea, oliguria and pruritus besides jaundice, and looked "increasingly toxic." Not enough details are given to permit a retrospective evaluation.

Case 19 of Nixon et al. was jaundiced from the 24th week until delivery at term in her first pregnancy. Jaundice appeared again in the 10th week of her second pregnancy. The liver was enlarged and total serum bilirubin rose to 10.5 mg per 100 ml. Two liver biopsies were performed both showing hyperplasia of the Kupffer cells and a fair amount of biliary pigment in the reticulum cells. The patient was not followed. In this case a hemolytic disorder or a subsiding viral hepatitis may be considered.

Case 11 of Boquien et al. was jaundiced during the 2nd, 3rd and 4th pregnancy. During the second pregnancy

eclampsia with hypertension and proteinuria occurred during the 8th month, followed by jaundice, convulsions, and delivery of a stillborn child by Caesarian section. During the 8th month of the 3rd pregnancy hypertension was observed and then delivery of a stillborn child took place in the presence of a retroplacental hematoma. Jaundice began at delivery and lasted for some weeks. Hemolysis was excluded. During the fourth pregnancy — again complicated by hypertension — pruritus and jaundice began one day after Caesarian section in the 8th month, lasting for 10 days. As jaundice began after delivery in the last two gestations intrahepatic cholestasis of pregnancy seems excluded. Eclampsia appears to have caused jaundice during the second and possibly also during the 3rd pregnancy.

The case reported by Justin-Besançon ¹¹ had onset of pruritus and jaundice during the first month of her first pregnancy associated with vomiting. She delivered at term and jaundice disappeared two weeks later. General health was markedly impaired and she lost 10 kg body weight during her gestation. During her second gestation jaundice and pruritus appeared in the 7th week after a prodromal phase of anorexia and vomiting. Because of impaired general condition and weight loss of 5 kg abortion was induced in the 12th week. This resulted in fever up to 38° centigrade and in an increase in jaundice. Twelve days later her serum bilirubin was 18.0 mg per 100 ml. Three weeks after delivery serum bilirubin was 3.5 mg per 100 ml with positive reactions to the thymol turbidity and the

zinc sulfate flocculation. Peritoneoscopy on the 31st day after delivery was unremarkable, bromsulfalein retention on the 37th day was 4.5 % after 45 minutes, a liver biopsy on the 43rd day (2 weeks after subsidence of jaundice) showed minimal cholestasis, and radiological gallbladder examination was normal. The general symptoms and weight loss, as well as the intensity of jaundice would point towards viral hepatitis as the cause of jaundice during the second gestation, but this cannot be conclusively proved.

Misquoted cases of recurrent jaundice during pregnancy in the literature

The case of Bjerregaard, published in 1904, had 3 normal pregnancies, then three with pruritus alone, followed by pruritus and jaundice during the 7th gestation only. This case is repeatedly cited as representing recurrent jaundice during pregnancy. In these quotations the author's name is usually misspelled and the bibliography incomplete or incorrect. More likely few reviewers bothered to have the Danish original translated.

Another case often quoted is one published in a French journal in 1907 by M. L. A. Meyer. The case report is truly an example of recurrent jaundice during pregnancy, but represents only a French abstract of a German paper by A. Mayer in 1906 with the author's name misspelled. Among the author's initials in the French abstract the *M* stands for *marquard* and the origin of the L. cannot be traced.

A case of Enrie et al. with tuberculoma of the liver has been called recurrent jaundice occasionally. This

She had a hematocrit of 36 % and a reticulocyte count of 6 %. Here, hemolysis appears to be an additional factor to whatever was the primary disease, which could have been chronic hepatitis or cirrhosis of the liver.

A similar case has been observed by Lebon et al. During the first two pregnancies this woman was jaundiced from the 7th month until shortly after her deliveries in the 8th month. During her third pregnancy she developed asthenia and pruritus in the 5th month, followed by intense arthralgias, back and chest pains, and then by jaundice. The liver was enlarged and tender, her general health markedly impaired, with a weight loss of 13 kg. Bilirubinuria was present, total serum bilirubin was 2.4 mg per 100 ml, alkaline phosphatase 15.8 Bodinsky units, the flocculation reactions normal, serum cholesterol low, and liver biopsy showed a granular degeneration of the liver cells, an increase in Kupffer cells and an increase in reticulum. This was interpreted as "periportal hepatitis." Her red cell count was 2,700,000 per cu mm and reticulocytes were 3.4 %, so that hemolysis appears superimposed to what was — from the history — viral hepatitis during her 3rd pregnancy. The episodes of jaundice during her first two pregnancies are not explained. Hypothetically it is possible that a woman has recurrent intrahepatic cholestasis during two pregnancies and then viral hepatitis during the third.

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The case described by Vignes in 1935 with jaundice during the 7th to 10th pregnancies has been considered to represent hemolytic jaundice by the author. The patient had tachycardia, dyspnea, oliguria and pruritus besides jaundice, and looked increasingly toxic. Not enough details are given to permit a retrospective evaluation.

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zinc sulfate flocculation. Peritoneoscopy on the 31st day after delivery was unremarkable, bromsulfalein retention on the 37th day was 45 % after 45 minutes, a liver biopsy on the 43rd day (2 weeks after subsidence of jaundice) showed minimal cholestasis, and radiological gallbladder examination was normal. The general symptoms and weight loss, as well as the intensity of jaundice would point towards viral hepatitis as the cause of jaundice during the second gestation, but this cannot be conclusively proved.

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Case II of Boquien et al. was jaundiced during the 2nd, 3rd and 4th pregnancy. During the second pregnancy

plete recovery in the anicteric intervals. It shall be briefly mentioned, mainly to show that its course differs markedly in all other respects from that seen in intrahepatic cholestasis of pregnancy. An other probably non-existing entity referred to in the older literature shall be listed to complete this survey.

Idiopathic recurrent cholestasis

Of this curious and only recently discovered disease only 12 cases have so far been described (Summerskill and Walshe 1959 2 cases, Tygstrup 1960 2 cases, De Groote et al 1960 11 cases, Kuhn 1960 2 cases, Schapiro and Isselbacher 1963 1 case and Williams et al 1964 3 cases). The entity consists of recurrent episodes of intrahepatic obstructive jaundice with complete clinical, functional and histological recovery in the intervals. Two patients are females and 10 are males. Two patients are brothers (Kuhn), two adolescent males are possibly related (Tygstrup) and in the other eight the family history is negative. First symptoms occurred between the ages of 1 and 29 years with 5 patients in the first 4 in the second and 3 in the third decade. The longest observed duration after the first attack of jaundice is 37 years with 27 episodes of jaundice recorded (Schapiro and Isselbacher).

An attack starts usually with a mild prodromal syndrome of anorexia and fatigue of up to 2 weeks duration. Marked pruritus and jaundice then set in lasting usually around 4 months with a range from 1 to 10 months. There is no fever or pain. Steatorrhea (up to 48 gm fecal fat excretion per day (Tygstrup case 2)) during jaundice leads to

a marked weight loss, despite good appetite. Weight loss is always regained in the asymptomatic interval. Jaundice is usually accompanied by moderate hepatomegaly. The spleen was slightly enlarged in two cases. Chemically, an obstructive pattern is observed. Serum bilirubin levels are usually between 8 and 23 mg per 100 ml, but may reach 40 mg per 100 ml. Serum alkaline phosphatase is mildly to moderately elevated, but may attain 94 King-Armstrong units. Serum cholesterol, however, is usually normal. It was mildly elevated in 2 cases (Tygstrup) and reached 480 mg per 100 ml in the case which also presents the highest recorded levels for serum bilirubin and alkaline phosphatase (Schapiro and Isselbacher). Serum transaminases have been normal or only slightly elevated. Serum turbidity and flocculation tests are normal as well as the albumin/globulin ratio. The erythrocyte sedimentation rate is slightly elevated and electrophoresis shows a mild increase in alpha 2 and beta globulins (Kuhn, Williams et al). Intravenous bilirubin tolerance tests show a regurgitation of conjugated bilirubin from the liver cells into the blood stream during jaundice but are normal in the anicteric interval (Williams et al). Gallbladder X-rays usually show no filling during jaundice but all intraoperative cholangiograms have been normal.

Liver biopsies during jaundice show marked centrilobular bile stasis with bile plugs in the canaliculi and bile pigment in the hepatocytes and in the Kupffer cells. In the centrilobular areas there is hepatocellular degeneration, loss of liver cells and inflammatory infiltration.

TABLE 23 Recurrent jaundice during pregnancy

-
- A Recurrent jaundice in pregnancy
- I Recurrent jaundice recurring also in non pregnant subjects
 - 1 Recurrent viral hepatitis or exacerbation of anicteric chronic hepatitis
 - 2 Recurrent jaundice in primary biliary cirrhosis
 - 3 Recurrent posthepatic hyperbilirubinemia
 - 4 Recurrent common bile duct obstruction due to gall stones
 - 5 Recurrent exacerbations of familial non hemolytic jaundice
 - 6 Recurrent hemolytic jaundice
 - 7 Recurrent jaundice with hemoglobinuria (?)
 - II Recurrent jaundice due to medical complications of pregnancy
 - 1 Recurrent jaundice in severe pyelonephritis
- B Recurrent jaundice of pregnancy
- I Recurrent idiopathic jaundice of pregnancy
 - 1 Recurrent intrahepatic cholestasis of pregnancy
 - II Recurrent jaundice as complication of disease linked to pregnancy
 - 1 Recurrent jaundice in hyperemesis gravidarum
- C Recurrent jaundice during pregnancy due to different diseases causing jaundice during pregnancy
Example Hepatitis in one hemolytic jaundice in the other gestation
- D Non classified cases of recurrent jaundice during pregnancy
-

woman had 3 episodes of jaundice during a single pregnancy (her fifth)

current jaundice during pregnancy before actual cases with adequate documentation are presented

4) Classification of recurrent jaundice during pregnancy

A classification of recurrent jaundice during pregnancy based on the cases described in chapter III/2 and III/3 is given in Table 23. The same general subdivisions are used as in Table 3 in the classification of (non recurrent) jaundice during pregnancy. It is theoretically possible, that other diseases listed in Table 3 and not listed in Table 23 could be recurrent, such as jaundice due to toxemia of pregnancy or jaundice due to tetracycline toxicity. We have refrained from listing them under re-

5) Other diseases with recurrent jaundice and complete recovery in the anicteric interval

Many patients with chronic liver disease such as chronic hepatitis or cirrhosis of the liver may run a course characterized by periods of exacerbations with jaundice and relatively symptom free anicteric intervals. Residual functional and/or structural damage persists, however, in these diseases. Apart from recurrent intrahepatic cholestasis of pregnancy only one disease is at present known with recurrent jaundice and com-

dice (Kehrer 1903), but some still denied a connection vehemently (Schickel 1910) or believed these cases to be due to gallstone obstruction (Rissmann 1910).

Gallstone obstruction of the common bile duct as a cause of cholestasis of pregnancy has been excluded since radiological examination of the gall bladder has been possible. Ilonen considers the incidence of coincidental gallstones not causing obstruction high in intrahepatic cholestasis of pregnancy (this opinion is based on non recurrent cases) and believes that gallstone formation could well be explained as a parallel phenomenon arising from the same disorder in metabolism which is causing the clinical picture of obstructive hepatitis.

With the recognition of viral hepatitis as one of the most common liver diseases during World War II the causal relationship between pregnancy and recurrent jaundice was questioned again and many, especially French authors considered the disorder to represent a recurrence or exacerbation of a previous viral hepatitis under the stress of pregnancy. The arguments for or against such a viewpoint have been summarized by Caroli et al. in 1954 who concluded that recurrent jaundice of pregnancy will but rarely be caused by viral hepatitis.

A type of latent familial non hemolytic jaundice with manifestation during pregnancy is suggested by the familial occurrence of intrahepatic cholestasis of pregnancy in at least 6 instances (see page 70). Gilbert's syndrome with an elevation of the indirect serum bilirubin

fraction only is ruled out immediately. Both the Rotor and the Dubin-Johnson syndrome have an elevation of the direct reacting bilirubin fraction. In the Dubin-Johnson syndrome pruritus is usually absent and the alkaline phosphatase not elevated. Liver biopsy with the characteristic blackbrown pigment is quite different from liver biopsy in intrahepatic cholestasis of pregnancy (Dubin). Liver biopsy in the Rotor syndrome is normal, as it is in some cases of intrahepatic cholestasis of pregnancy. Laboratory findings are somewhat similar, but bromsulfalein retention is more pronounced and oral contrast materials visualize the gallbladder well. The patients with the Rotor syndrome are asymptomatic and do not have pruritus (Schiff et al., Peck et al.). Furthermore jaundice decreases during pregnancy (Haverback and Wirtschafter).

The concept of bile stasis was probably first advanced by Boreel in 1924. It was clearly expressed by Perreau in 1933 and by Svanborg in 1934. Thorling in 1935 proposed the concept of incomplete intrahepatic biliary tract obstruction precipitated by hepatic damage. The main theories advanced to explain the mechanism of intrahepatic cholestasis in this disorder are atony of the extrahepatic biliary passages (Svanborg), change in the chemical composition of bile leading to inspissation (Ljunggren) and a change in membrane permeability at the level of the bile capillaries (Gros). The first two possibilities appear unlikely because of the observation of normal bile drainage from a T tube in the case of Beraud et al. This provides of course no evidence in

tion Some portal tracts show edema or infiltration (Williams et al.) Biopsies in anicteric intervals are normal or may show minimal residual bile stasis. Laparotomies and peritoneoscopies have revealed a brownish or greenish discoloration of the liver but otherwise normal findings.

Both of the 2 reported women have been pregnant during the course of their disease. The case with 27 episodes of jaundice during age 3 and 40 underwent a single normal anicteric pregnancy at age 25 (Schapiro and Isselbacher). The other woman had her first episode of jaundice in the second month of her first pregnancy. After an artificial abortion in the fourth month of gestation the jaundice persisted. Three further pregnancies were anicteric during the whole course.

Thus, pregnancy does not induce a relapse of jaundice in this disease.

Recurrent jaundice during menstruation

In 1872 Senator described 4 women who developed jaundice for a few days during each successive menstrual bleeding. A careful review of his case reports does not support his enthusiastic association of jaundice with menstruation. The maximal episodes of jaundice in a single patient were six, with anicteric menstruations or—in one case—a normal pregnancy thereafter. Furthermore the time relationship is not always convincing. During the 'disease' jaundice is said to occur instead of a missed menstrual bleeding. From the data presented it is impossible to diagnose the nature of this jaundice. A fifth case in the litera-

ture, reported by Metzger in 1904, is clearly a description of an acute viral hepatitis with several relapses.

No further such cases have been described and it must be concluded that 'recurrent jaundice during menstruation' does not exist.

6) Pathogenesis of intrahepatic cholestasis of pregnancy

A review of the older literature concerning speculations on the pathogenesis of recurrent jaundice during pregnancy has been presented by Mayer in 1906. The prevalent idea at that time was a mechanical compression of the extrahepatic bile ducts by the enlarged uterus pressing on a constipated transverse colon. This mirrors the views held by the authors of textbooks on liver disease in the 19th century (Budd and Hensch 1846, French 1858, Quincke and Hoppe-Seyler 1899). Other theories on the pathogenesis of benign jaundice during pregnancy included "nervous influences", 'plethora of organs during pregnancy', "dyspepsia", "gastroduodenitis", a 'mucous plug in the common bile duct' and "sudden emotions" (Meunier 1872).

A similar line of thought is pursued by Roumanian authors in 1957 (Pavel et al.) who assume that distension of the peritoneum overlying the enlarging uterus triggers nervous reflexes which then will—modified by a terrain of hyperfolliculinemia—lead to a spasm of the sphincter of Oddi and perhaps to reflex inhibition of bile secretion.

Early in the 20th century most authors were convinced of a causal relationship between pregnancy and recurrent jaun-

dice (Aehrer 1905), but some still denied a connection vehemently (Schickel 1910) or believed these cases to be due to gallstone obstruction (Rissmann 1910)

Gallstone obstruction of the common bile duct as a cause of cholestasis of pregnancy has been excluded since radiological examination of the gall bladder has been possible. Ikonen considers the incidence of coincidental gallstones not causing obstruction high in intrahepatic cholestasis of pregnancy (this opinion is based on non recurrent cases) and believes that gall stone formation could well be explained as a parallel phenomenon arising from the same disorder in metabolism which is causing the clinical picture of obstetric hepatitis

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favour of the membrane permeability theory

Whenever the etiology of a disease is unknown, one is inclined to draw analogies to similar diseases. The syndrome intrahepatic cholestasis includes (apart from pregnancy) viral hepatitis, drug induced cholestasis, acute fatty liver and alcoholism (Dolle and Martini 1959 and 1962, Jeffries and Sleisenger).

Fatty liver and alcoholism can readily be excluded on the basis of liver biopsy findings and personal history in patients with intrahepatic cholestasis of pregnancy.

Viral hepatitis may occur under the form of pure intrahepatic cholestasis without signs of inflammation or liver cell damage on liver biopsy (Caroli et al 1953, Dubin et al 1960, histological type D). These cases are extremely rare (6 patients in these two papers) and present clinically the usual prodromal symptoms seen in typical acute viral hepatitis which are absent in intrahepatic cholestasis of pregnancy. More frequent are forms of viral hepatitis with obstructive features (so-called cholangiolitic hepatitis) with findings of typical viral hepatitis on biopsy (Watson and Hoffbauer, Gall and Braunstein, Dubin et al, histological types B and C) so that they are easily distinguishable from intrahepatic cholestasis of pregnancy.

Primary biliary cirrhosis may in the initial stages present with pruritus without jaundice with an elevation of alkaline phosphatase and an elevation of bromsulphalein retention (Popper et al 1962). No case of recurrent cholestasis of pregnancy has yet progressed to

primary biliary cirrhosis. The case reported by Lylecote probably represented primary biliary cirrhosis from the onset of its clinical course.

Drug induced cholestasis is most likely to induce comparison with intrahepatic cholestasis of pregnancy especially as sex hormones can be implicated in both disorders. In addition Read et al suggested that pregnant women may be especially susceptible to the development of chlorpromazine induced jaundice although this has not been confirmed by others (see page 32).

Drug induced intrahepatic cholestasis consists of two main types: a sensitivity type (example chlorpromazine jaundice) which histologically shows portal infiltrations in addition to cholestasis and a non sensitivity type (example norethandrolone jaundice) with the histological picture of pure cholestasis (Sherlock). The latter type is histologically comparable to intrahepatic cholestasis of pregnancy. It is produced by methyl testosterone and other C 17 α alkyl substituted testosterone such as norethandrolone (Nilevar®), methandienone (Dianabol®) and the ovulation inhibitor Enavid® which contains norethynodrel.

In this connection an interesting observation has been made in case L. A reported by Ikonen. This woman with intrahepatic cholestasis of pregnancy in 2 successive gestations was given an ovulation inhibitor containing norethisterone and aethinylloestradiol 9 1/2 months after the last delivery. She developed abdominal pains, nausea and — after one week — dark urine and pruritus followed by jaundice and an increase in

SGOT The drug was stopped and jaundice disappeared. The patient later received progesterone without ill effect.

King and Kerrins, in their report on a case with recurrent intrahepatic cholestasis of pregnancy, considered this disorder to have a striking resemblance to drug induced jaundice. Similar opinions have been voiced by Swanborg and Ohlsson and by Gros. Such statements have to be firmly refused. Although the case of Ilonen suggests that pregnancy and the ovulation inhibitor triggered the same disease process and although histology in norethandrolone-type cholestasis is indistinguishable from intrahepatic cholestasis of pregnancy, there are important and mutually exclusive differences between the two disorders. Jaundice in intrahepatic cholestasis of pregnancy is always mild and is not known to have surpassed a serum bilirubin level of $8 \pm \text{mg per } 100 \text{ ml}$. In drug induced cholestasis jaundice is mild in the majority of cases, but may reach levels of up to $30 \text{ mg per } 100 \text{ ml}$ of serum bilirubin (Werner et al). In norethandrolone type jaundice alkaline phosphatase is comparatively little elevated. Furthermore a prodromal period with anorexia, malaise and often fever is common in drug induced jaundice (Schaffner) and was present in the ovulation inhibitor induced jaundice in Ilonen's case while prodroms are absent in intrahepatic cholestasis of pregnancy. In addition pruritus is the dominant symptom in intrahepatic cholestasis of pregnancy while in drug induced jaundice pruritus is present in only about half the cases. More significant even, methyl testosterone and norethandrolone are ef-

fective therapeutic agents to relieve pruritus of hepatic origin, although they increase the intensity of pre-existing jaundice at the same time.

A disordered hormonal balance remains an attractive hypothesis for the basic pathogenetic mechanism in intrahepatic cholestasis of pregnancy, but such a hypothesis should not be founded on a comparison of this disease with steroid induced jaundice as we know it at present.

It might be worth while to consider briefly the meaning of the term "intrahepatic cholestasis". Cholestasis has been given two basic definitions: a clinical-functional one and a histological one. Clinically and functionally cholestasis is defined as a disease with pruritus, an elevation of serum alkaline phosphatase and often an elevation of serum cholesterol. Histologically cholestasis is defined as the presence of bile plugs (or bile thrombi) in the bile canaliculi (or bile capillaries), and the presence of bile pigment in hepatic cells and Kupffer cells predominantly in the centrilobular area (Popper and Schaffner). While in most examples of intrahepatic cholestasis functional and histological cholestasis are present more or less parallel to one another in others they are not and functional or histological cholestasis may be completely absent. On one end of this spectrum lies the entity called post-operative intrahepatic cholestasis which presents a marked and pure cholestasis on liver biopsies but functionally predominantly an elevation of serum bilirubin of up to $27 \text{ mg per } 100 \text{ ml}$ with normal or mildly elevated alkaline phosphatase and absence of pruritus (Schmid

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Whenever the etiology of a disease is unknown, one is inclined to draw analogies to similar diseases. The syndrome "intrahepatic cholestasis" includes (apart from pregnancy) viral hepatitis, drug-induced cholestasis, acute fatty liver and alcoholism (Dolle and Martini 1959 and 1962 Jeffries and Sleisenger)

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- 12 Drainage of bile from a T tube in the common bile duct is normal during jaundice
- 13 Premature delivery is frequent, but limited to some women, while about two thirds of the patients deliver at term. There is no correlation between premature delivery and any other feature of the disorder
- 14 The intensity of symptoms during successive pregnancies with jaundice may remain equal, may increase or may decrease
- 15 Recurrent pruritus gravidarum may be observed before recurrent pruritus with jaundice in some cases

At present no pathogenetic explanation based on facts can be offered at least none giving an insight into what ever process launches the disorder. It appears reasonable however to assume that — whatever this triggering process is — *physiological derangement of liver function in normal pregnancy, pruritus gravidarum and intrahepatic cholestasis of pregnancy are but three increasing*

grades of manifestation of the same basic disorder linked in some way to pregnancy (Friedberg 1951)

In uncomplicated pregnancies there is towards term an increase in alkaline phosphatase, serum cholesterol, serum lipids alpha and beta globulins and bromsulfalein retention, and a decrease in bromsulfalein excretory capacity (BSB T_m) and intravenous bilirubin tolerance. In pruritus gravidarum these same alterations are more pronounced and additionally the serum transaminases are mildly elevated. Intrahepatic cholestasis of pregnancy shows again an increase of the disturbances seen in pruritus gravidarum with the addition of an elevated serum bilirubin, a radiologically non visualized gallbladder and histological evidence of intrahepatic cholestasis.

Thus intrahepatic cholestasis of pregnancy is best viewed as an exaggeration of the alterations of liver function occurring to a minor degree also in uncomplicated pregnancies.

et al) Intrahepatic cholestasis of pregnancy represents the other end of this spectrum, as in this disorder pruritus is violent, biochemical cholestasis usually impressive and histological cholestasis focal and minimal, so minimal that it can be easily missed by the pathologist. At present no explanation can be offered for this divergence of functional and structural cholestasis, but this aspect should be kept in mind when pathogenesis is discussed.

An explanation of the pathogenesis of recurrent intrahepatic cholestasis of pregnancy has — in order to be of value — to explain all of the following features in this disorder:

- 1 The disorder is strictly linked to pregnancy and does not occur in non pregnant subjects
- 2 The disorder usually presents as 'jaundice during late pregnancy' although onset of jaundice may be as early as in the first trimester in some cases
- 3 Pruritus is the first symptom to occur and the main symptom during the course of the disease
- 4 Apart from pruritus there are no prodromal symptoms. There is a conspicuous absence of general symptoms such as fever, weakness, malaise, anorexia, nausea, vomiting, dyspepsia, pains, colics, arthralgias, weight loss
- 5 Jaundice is always of mild degree. The highest observed serum bilirubin level is 8.4 mg per 100 ml. In most cases it is below 6 mg per 100 ml. Direct reacting bilirubin constitutes the main portion of total serum bilirubin elevation. Bilirubinuria is transient or intermittent, but probably present in all cases
- 6 Serum alkaline phosphatase and serum cholesterol are elevated in most and serum transaminases in many cases. Any of these three parameters may occasionally be normal in a single patient. Highest observed values are alkaline phosphatase 28 Bodansky units, serum cholesterol 590 mg per 100 ml, SGOT 920 units, SGPT 875 units (although transaminases are with few exception below 250 units). Serum electrophoresis shows an increase in alpha 2- and mainly in beta globulins, with normal or slightly decreased gamma-globulins. There is no correlation between any two laboratory tests
- 7 Bromsulfalein retention is increased and galactose tolerance is normal. Bromsulfalein clearance studies reveal a decreased hepatic storage capacity and an increased cholestatic index
- 8 Rapid improvement sets in after delivery, whether delivery is spontaneous or induced
- 9 In closely followed cases there may be a transient return to normal of serum bilirubin and of transaminases on the first postpartum day
- 10 Recovery after delivery is complete. No permanent liver damage ensues after multiple pregnancies with jaundice
- 11 Histological cholestasis on liver biopsy is mild and focal or irregular. This is in marked contrast to the impressive clinical and biochemical signs of cholestasis

- 12 Drainage of bile from a T tube in the common bile duct is normal during jaundice.
- 13 Premature delivery is frequent, but limited to some women, while about two thirds of the patients deliver at term. There is no correlation between premature delivery and any other feature of the disorder.
- 14 The intensity of symptoms during successive pregnancies with jaundice may remain equal, may increase or may decrease.
- 15 Recurrent pruritus gravidarum may be observed before recurrent pruritus with jaundice in some cases.

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Thus intrahepatic cholestasis of pregnancy is best viewed as an exaggeration of the alterations of liver function occurring to a minor degree also in uncomplicated pregnancies.

The main purpose of this review is to define the entity called "recurrent intrahepatic cholestasis of pregnancy" and to establish a differential diagnosis on the many disorders which may present as recurrent jaundice during pregnancy.

PART I contains a review on "liver function" during uncomplicated pregnancy. The liver performs its function well during gestation, but most so called "liver function tests" show some minor deviations from the accepted normal in non-pregnant subjects. These "physiological" derangements are more common in the later weeks of pregnancy and are rapidly rectified after delivery.

Liver biopsy findings remain generally within normal limits. Some minor nonspecific changes may occur, such as a difference in size of liver cells, an increase in size of their nuclei, some irregularities of the nuclei, an increase in binucleated cells, an increased glycogen content of the cytoplasm and occasional mild lymphocytic infiltrations in the portal tracts.

Spider angiomas and palmar erythema may be found in up to two thirds of pregnant females. These skin changes are rarely impressive.

Liver blood flow remains quantitatively unchanged. As plasma volume and total blood volume increase by about

50 % in normal pregnancy relative liver blood flow (as fraction of cardiac output) decreases somewhat.

Hemoglobin falls as a result of increasing plasma volume. Serum iron fluctuates, but remains on the whole constant. Total white cell counts may be increased to up to 15,000 per cu mm and a "shift to the left" is not uncommon.

Alkaline phosphatase increases towards term, with usually a pronounced rise after the 7th month of gestation. Serum cholesterol and serum lipids follow the same trend. On electrophoresis a slight increase in alpha 2 and beta globulins is noted. These changes, though mild, are indicative of an "obstructive pattern" even in normal pregnancy.

Serum bilirubin may in a rare case be elevated to up to 2 mg per 100 ml and abnormal urinary bile pigments may be occasionally present. These changes are not related to the stage of pregnancy in contrast to the "obstructive features" which tend to increase towards term.

Total serum proteins and serum albumin decrease towards term. The flocculation and turbidity tests are usually normal, but positive results are seen in varying proportions of cases, probably dependent more on the technique used in a specific laboratory than on the test itself.

Bromsulfalein retention is slightly in-

creased towards term, due to an increase in hepatic storage capacity and a decrease in maximal excretory capacity. Galactose tolerance remains normal.

The only liver function tests to remain normal throughout pregnancy are the serum transaminases and the prothrombin time.

With the exception of the changes in the white cell count most deviations from the normal are minor. Main diagnostic difficulties will be encountered with the alkaline phosphatase and the turbidity or flocculation tests.

PART II contains a review on all diseases causing jaundice during pregnancy. Jaundice occurs in about 1 out of every 1500 gestations, an incidence of 0.067%. At least 41% of all cases with jaundice are due to viral hepatitis, and about 21% due to intrahepatic cholestasis of pregnancy. Common bile duct obstruction accounts for less than 6% of all cases with jaundice.

A classification of jaundice during pregnancy is proposed in Table 3.

A first group of diseases "jaundice in pregnancy" consists of entities seen also in non pregnant persons occurring by chance during gestation.

Viral hepatitis during pregnancy has often been quoted to run a severe course during pregnancy. A review of the literature reveals that pregnant women are not more susceptible to viral hepatitis than non pregnant subjects; that viral hepatitis runs the same course in pregnancy as outside of it and that mortality from viral hepatitis is not increased during pregnancy at least not in Europe.

An exception to this rule may be seen

in the indigene population of underdeveloped countries, where malnutrition and general debility provide a serious hazard to any additional injury during gestation. Viral hepatitis occurs probably with equal frequency during all trimesters of gestation and not predominantly towards term, as has been frequently stated. The course of severe hepatitis is not influenced by an induced interruption, and termination of pregnancy is not advised. Hepatitis induces an increased incidence of premature deliveries. Child survival depends on the degree of maturity. No conclusive evidence exists that viral hepatitis can be transmitted from the mother to the unborn child at least not in the later part of gestation.

Pregnancy occurs rarely during cirrhosis of the liver because fertilization is impaired in this disease. For this reason, cirrhotics who do get pregnant probably represent a selection of the less severe cases and generally tolerate gestation surprisingly well even when they previously had manifestations of decompensated liver function. Pregnancy increases intra abdominal pressure and probably also pre-existing portal hypertension. A rare case will develop ascites. Occasionally hematemesis from esophageal varices is induced. Successful porto-caval shunts have been performed during pregnancy. Hematemesis towards term may be stopped by a Caesarian section. Child survival is not affected by the mother's cirrhosis.

Drug induced intrahepatic cholestasis (usually due to chlorpromazine) is probably not influenced by pregnancy although 4 of 22 chronic cases in the

literature began in pregnancy, including the case with the longest recorded duration of 3 years before complete recovery. The prodromal phase is similar to that seen in viral hepatitis, the biochemical data show an "obstructive pattern".

Gallstone disease is — contrary to general opinion — in most likelihood not caused by nor increased during pregnancy and common bile duct obstruction is rare during gestation. It is handled according to general medical or surgical principles.

Data on the behaviour of chronic idiopathic hyperbilirubinemias are sparse. Pregnancy generally aggravates the intensity of jaundice in the Dubin—Johnson syndrome, may lessen it in the Rotor syndrome, and does usually not influence the course in Gilbert's syndrome.

Hemolytic disorders during pregnancy usually present as marked anemia and an accompanying jaundice is generally mild. The primary hemolytic disorders during pregnancy — megaloblastic anemia of pregnancy due to folic acid deficiency and idiopathic hemolysis of pregnancy — are exceedingly rare. Pre-existing chronic hemolytic states may be aggravated during gestation, such as familial spherocytosis and some hemoglobinopathies, especially S—C, S—S and C—C disease. The combination of hemoglobinopathies and pregnancy is hazardous for both mother and child. Secondary hemolysis may be observed after incompatible blood transfusions and in overwhelming septicemias.

Jaundice occurring in severe pyelonephritis during pregnancy (probably due to septicemia) has nearly disap-

peared since the advent of the antibiotics. A new syndrome has replaced it: jaundice in tetracycline toxicity used in the treatment of pyelonephritis during pregnancy. This toxic liver damage is often fatal. Postmortem examination reveals a diffuse fatty metamorphosis of the liver. So far it has not been seen in nonpregnant subjects.

Jaundice due to delayed chloroform poisoning and jaundice in criminal abortions are mainly of historic interest.

A second group of diseases, "jaundice of pregnancy", consists of disorders caused by pregnancy itself or occurring in typical complications linked to pregnancy.

Intrahepatic cholestasis of pregnancy is an entity benign to both mother and child, but has a marked tendency to recur in successive gestations. There are no prodromal symptoms such as in viral hepatitis or as in drug-induced intrahepatic cholestasis. The disease is characterized by often violent pruritus and mild jaundice, both disappearing usually within two weeks after spontaneous or induced delivery. Onset of jaundice is generally after the 22nd week of gestation but may occur as early as the 7th week. Biochemically there is an obstructive pattern with an increase of serum bilirubin, alkaline phosphatase, cholesterol, and alpha 2 and beta globulins on electrophoresis. Transaminases are mildly to moderately increased. Abnormal bromsulfalein retention is present but galactose tolerance is normal. Flocculation and turbidity reactions are normal. Radiological gallbladder examinations show no filling during jaundice but bile flow in the extrahepatic biliary

system is unimpaired Liver biopsy shows only a mild and usually focal or irregular cholestasis with no evidence of liver cell damage

Acute fatty metamorphosis of pregnancy is a rare disorder with a very high mortality Histologically it presents as a diffuse fatty change of the liver with the exception of a sharply defined rim of normal liver cells around the portal tract There is no necrosis or inflammation Clinically the disease resembles fulminant viral hepatitis Onset of symptoms is always after the 30th and in the majority after the 36th week of gestation Sudden and persistent vomiting is followed by abdominal pains jaundice and tachycardia There is no fever The patient rapidly becomes somnolent and vomiting assumes a coffee ground aspect Premature labor sets in the patient delivers a stillborn child lapses into coma and dies with a terminal temperature elevation usually 1 to 7 days post partum Few patients have survived Two survivals occurred after early Caesarian section Serum bilirubin rarely surpasses 10 mg per 100 ml alkaline phosphatase and transaminases are moderately elevated prothrombin time is markedly prolonged white cell counts vary between 20 000 and 30 000 and flocculation and turbidity reactions are normal Hypoglycemic episodes may occur and oliguria with azotemia is not uncommon A few patients had associated pancreatitis

Both intrahepatic cholestasis of pregnancy and acute fatty metamorphosis of pregnancy do not occur outside of gestation and in both the etiology is unknown

Hyperemesis gravidarum is occasionally associated with mild jaundice and rarely with pruritus Pathological bile components may be present in the urine, the transaminases may be slightly elevated and occasionally the flocculation tests are positive Histological liver changes are absent or nonspecific Jaundice in hyperemesis has no prognostic significance and clears with the cessation of vomiting

Toxemia of pregnancy is associated with a high incidence of abnormal liver function tests Flocculation tests are positive in about half the cases Alkaline phosphatase is elevated and parallels the clinical course Transaminases are increased in the more severe cases Jaundice is rare and carries a grave prognostic outlook Histological liver changes are impressive at postmortem examination but absent or minor in liver biopsies These changes appear to be a terminal event reflect the basic vascular disorder and cannot be implicated in the genesis of the disturbed liver function tests

The only disease in which an induced termination of pregnancy appears indicated because of jaundice is acute fatty metamorphosis of pregnancy In order to save the patient it should be performed very early after the onset of symptoms An interruption of pregnancy may become necessary in toxemia but indications are based on general obstetric principles Interruption of pregnancy may also be indicated in some cases of hemolysis not responding to medical treatment but the decision is based on the degree of anemia and not on the presence of jaundice Interrup-

literature began in pregnancy, including the case with the longest recorded duration of 3 years before complete recovery. The prodromal phase is similar to that seen in viral hepatitis, the biochemical data show an "obstructive pattern"

Gallstone disease is — contrary to general opinion — in most likelihood not caused by nor increased during pregnancy and common bile duct obstruction is rare during gestation. It is handled according to general medical or surgical principles.

Data on the behaviour of chronic idiopathic hyperbilirubinemias are sparse. Pregnancy generally aggravates the intensity of jaundice in the Dubin—Johnson syndrome, may lessen it in the Rotor syndrome, and does usually not influence the course in Gilbert's syndrome.

Hemolytic disorders during pregnancy usually present as marked anemia and an accompanying jaundice is generally mild. The primary hemolytic disorders during pregnancy — megaloblastic anemia of pregnancy due to folic acid deficiency and idiopathic hemolysis of pregnancy — are exceedingly rare. Pre-existing chronic hemolytic states may be aggravated during gestation, such as familial spherocytosis and some hemoglobinopathies, especially S—C, S—S and C—C disease. The combination of hemoglobinopathies and pregnancy is hazardous for both mother and child. Secondary hemolysis may be observed after incompatible blood transfusions and in overwhelming septicemias.

Jaundice occurring in severe pyelonephritis during pregnancy (probably due to septicemia) has nearly disap-

peared since the advent of the antibiotics. A new syndrome has replaced it: jaundice in tetracycline toxicity used in the treatment of pyelonephritis during pregnancy. This toxic liver damage is often fatal. Postmortem examination reveals a diffuse fatty metamorphosis of the liver. So far it has not been seen in nonpregnant subjects.

Jaundice due to delayed chloroform poisoning and jaundice in criminal abortions are mainly of historic interest.

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tion is definitely not indicated in intrahepatic cholestasis of pregnancy, drug-induced cholestasis, familial hyperbilirubinemias, pyelonephritis and tetracycline toxicity. The course of viral hepatitis and liver cirrhosis is not altered by interruption of pregnancy and surgery is poorly tolerated in the severe cases in both diseases. Jaundice due to common duct stones is handled according to usual medical and surgical principles.

PART III contains a review on recurrent jaundice during pregnancy. A total of 132 cases are reported in the world literature. For the purpose of this review the cases have been divided into 6 categories according to their documentation (see Table 10). In 43 cases the diagnosis of intrahepatic cholestasis of pregnancy has been accepted (categories I and II). In 28 cases a different disease entity is responsible for recurrent jaundice (category VI). In 61 cases documentation is insufficient for a critical evaluation (categories III—V). In addition, 267 cases of non-recurrent intrahepatic cholestasis of pregnancy are briefly mentioned (category VII).

The terminology formerly used for recurrent intrahepatic cholestasis of pregnancy includes icterus gravidarum, jaundice of pregnancy, idiopathic jaundice of pregnancy, recurrent jaundice of pregnancy, benign jaundice of pregnancy, idiopathic hepatopathy of pregnancy, obstetric hepatosis, endogenous hepato toxemia of pregnancy, and cholestatic jaundice of pregnancy.

In 14 reports covering 23 patients the diagnosis of intrahepatic cholestasis of pregnancy is based on liver biopsies and

can be accepted beyond reasonable doubt (category I). Adequate laboratory examinations and details on clinical course are reported in 18 of these cases (category I A). These patients underwent a total of 70 gestations, in 47 of which the full syndrome with pruritus and jaundice occurred. The 47 pregnancies form the basis for establishing criteria of diagnosis in this disease.

Liver biopsies reveal only a mild centroacinar cholestasis, which is focal or irregular. Some bile canaliculi contain bile plugs. They are of normal caliber or slightly dilated. The surrounding liver cells contain biliary pigment. The liver cells are intact, as are the portal fields. There is no inflammation. Minor changes are consistent with those seen in uncomplicated pregnancies. Macroscopical findings in 10 patients undergoing surgical laparotomies were considered normal. Only one patient had gall bladder stones. In none was extrahepatic bile flow impaired. Radiological gallbladder examinations show no filling during jaundice and are normal after its subsidence.

Pruritus precedes jaundice usually by 1 to 2 weeks, in rare instances by up to 22 weeks. Itching is violent, involves the trunk and/or the extremities and leads to insomnia. Mean onset of jaundice is in the 26th week with an observed range between the 7th and the 39th week. Median duration of jaundice from its onset to spontaneous delivery is 6 weeks, and mean duration 8 1 weeks with an observed range from 1 to 33 weeks. There are no prodromal symptoms such as in viral hepatitis. Apart from itching there are no general symp-

ptoms General well being is not impaired Physical examination is normal except for the presence of jaundice and scratch marks After delivery, whether spontaneous or induced jaundice disappears within 1 to 2 weeks in the majority of patients, but may last in some up to 4 weeks postpartum Itching subsides before jaundice Recovery is complete after delivery

Peak serum bilirubin is below 8.4 mg per 100 ml in all and below 6 mg per 100 ml in most cases The direct reacting bilirubin constitutes the major fraction Urobilinogen is never absent from the urine Urobilin and bilirubin are usually present Bilirubinuria may be transient and can be missed Alkaline phosphatase is increased to peak levels of 28 Bodansky units and serum cholesterol to maximally 290 mg per 100 ml One or both these parameters may be normal in an occasional case Serum electrophoresis shows an increase in alpha 2 and beta globulins and a decrease in serum albumin Gamma globulins are always lower than beta globulins Flocculation and turbidity test are normal Prothrombin time may be prolonged when jaundice is of long duration This is due to a deficiency in the Vitamin K dependent coagulation factors II, V, VII and X Serum transaminases are elevated up to 250 units and reached 400 units in a single instance Brom sulfalein retention at 45 minutes is between 10 and 25 % Galactose tolerance is normal There is no evidence of hemolysis

After delivery there is a rapid decline of serum bilirubin and serum transaminases while alkaline phosphatase

may continue to rise for 4 to 10 days post partum There are no obstetrical complications In cases with prolonged prothrombin time blood loss during delivery may be excessive The overall incidence of premature deliveries is high Interestingly enough, premature delivery is confined to about one third of the patients while the others deliver at term Premature delivery appears to be independent of jaundice per se It may be caused by the same metabolic disturbance which also causes intrahepatic cholestasis Child survival depends on the degree of maturity No baby was icteric

In successive pregnancies the syndrome may increase or decrease in severity or may present repeatedly with the same intensity Clinical course regarding onset of jaundice and date of delivery is similar in many patients

Treatment consists in the prophylactic application of Vitamin K and in the administration of cholestyramine to relieve itching Neither diet nor bed rest are necessary and the patient may continue her usual daily life

In some instances there are 2 or more cases of recurrent intrahepatic jaundice of pregnancy in close relatives No antecedent liver disease is responsible for its occurrence

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The differential diagnosis of recurrent jaundice during pregnancy is given in Table 23. Recurrent jaundice during pregnancy may probably occur in all benign disorders causing jaundice during pregnancy. Documented examples have been published for hemolytic jaundice, familial non hemolytic jaundice, post hepatic hyperbilirubinemia, gall stone obstruction of the common bile

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The diagnosis of intrahepatic cholestasis of pregnancy should only be made when the criteria outlined above are met and when the differential diagnosis of recurrent jaundice during pregnancy has been carefully considered.

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IN HONOUR OF
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DEDICATED PAPERS ON THE OCCASION
OF HIS SIXTIETH BIRTHDAY
APRIL 17 1966

LUND 1966

**REDIGENDA CURAVIT
SVEN LRIK BJÖRMAN**

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IAN WALDENSTROM

APRIL 17 1966

Jan Waldenstrom is professor of a medical faculty of the third generation. His grandfather Johan Waldenström was professor of internal medicine in Uppsala and his father Henning Waldenström is retired as professor of orthopedic surgery in Stockholm. Jan Waldenström, who has become a firm believer in the importance of genetic factors in metabolic diseases, is thus himself a living evidence that the destiny of man is ruled by his genes.

Medicine of today and particularly internal medicine is faced with vast problems. Rapid progress has created a state of flux. It is impossible to predict or even guess the future course of medicine. There is a tendency towards division into smaller and more specialised branches. This has created an urgent need for men equipped with receptiveness to the complete multiplicities of human experience coupled with polymathic knowledge of past and modern medical science and at the same time with the ability to set up synopses for new approaches to diagnostic and therapeutic problems.

What is required by such a man? A comprehensive and vigilant brain, an apprehensive and open scientific mind, a broad and keen interest in human behaviour, sharpness of observation, a selective pigeon-holed memory and intrepidity.

Jan Waldenström has these qualities — by inheritance. During the last thirty years he has entered widely separated fields of medicine to all of which he has contributed with original observations of lasting value. His work has greatly influenced not only the research work of his contemporaries but also the march of medicine in general.

None the less it would be wrong to think that he has not followed his own line. As a young doctor he was given the unique opportunity of studying organic chemistry in the laboratory of Hans Fischer in Munich, the pyrrole pigment chemist and Nobel prize winner. This biochemical background has

Of utmost importance for these studies was the unique opportunity Waldenström had in Uppsala to collaborate with members of the Institute of Physical Chemistry. In collaboration with Pedersen sedimentation constants and electrical mobility of serum proteins in different diseases with elevated ESR were determined by means of The Swedberg's ultracentrifuge and the Tiselius electrophoresis method. It is not presumptuous to state that the foundation stone of the present surge of interest in serum protein disturbances was laid there.

Waldenström recognized in the early 1950s the qualitative difference between the increased globulins in myeloma and diseases due to chronic infection or collagen states, namely the electrophoretically narrow and broad peak gamma type. He concluded that in myeloma only a part of the gamma fraction was increased in contrast to the broad gamma peak. This fundamental observation led him logically ten years later to coin the names monoclonal and polyclonal for these hypergammaglobulinemias. By that time it had become apparent that there also existed a clinically benign type of narrow peak gamma not associated with myeloma. So now the follow up of patients with an elevated ESR proceeds as a follow up of patients with narrow bands in order to settle the clinical importance of this finding. In this connection he has also coined the name gammopathies and stressed the importance of hereditary factors in the causation of gamma disorders. He infers that the synthesis of gammaglobulins is genetically controlled. Another example of this is the change of the gamma synthesis in the opposite direction seen in the hereditary sex linked agammaglobulinemia first described by the master of gammopathies, Jan Waldenström.

For 25 years Waldenström has had a never waning interest in the myeloma disease not only from the point of protein disturbance but also with respect to its clinical manifestations, cellular morphology, prognosis and treatment. He stands here as always as a link otherwise often missing between the theoretical laboratory research workshop and the practical clinical work. His experience of the biological nature of this malady is eminent and his therapeutic program for cytostatic treatment is probably one of the most promising in malignant disorders.

Somebody once stated that the compilation of a new syndrome should be a rather simple task for the experienced clinician. In these days of computer analysis it requires perhaps even less effort. Waldenström's conception of the

resulted in his adopting a metabolic approach to clinical medicine. He feels that every disorder is basically an interplay of biochemical reactions. He advocates that it is the diagnostic goal to find out what material it is in the sick body that is in excess or deficient as a result of the metabolic disturbance, analogous to the accumulation of porphobilinogen in the urine in acute porphyria.

The first example of his biochemical approach to clinical medicine is his classical monograph »Studien über Porphyrine» (Uppsala 1937), in which acute porphyria for the first time was shown to be a hereditary disorder of porphyrin synthesis which was manifested biochemically by the excretion of uroporphyrin III in the urine. This and other works of his brought order into the controversial state of the porphyrias and clarified our concepts. It has awarded him a rank of honour as a pioneer in clinical porphyrin research.

With his keen interest in pyrrrol chemistry it is not surprising that hematology, particularly iron metabolism, became a new field of research. A series of articles on hematological subjects followed his porphyrin works. Among those large population studies on the incidence of iron deficiency anemia were pioneer work, clearly before its time. He was also one of the first to perform iron balance studies long before isotope techniques had been developed. Pulmonary hemosiderosis was first described by him as a new clinical entity and with a peculiar type of iron deficiency. Sideropenic dysphagia was reevaluated and the roentgen diagnostic procedure was improved. Iron deficiency problems have always since been among his favourite subjects and he was the first to describe symptoms of iron deficiency without anemia.

While studying these subjects he also began his research in serum proteins. I think that the constellation in Uppsala was particularly favourable for such studies at that time. The use of the erythrocyte sedimentation rate (ESR) in clinical medicine became a routine procedure in Sweden in the early 1930s. Waldenström's interest in serum proteins emerged from follow up studies of patients with constantly elevated ESR. These patients were mostly members of farmer families from the rural neighbourhood of Uppsala who regularly went to town to do their business and shopping and to have their ESR measured. Without their knowledge their initials flew all over the world carrying with them new ideas and names of new disease concepts — purpura, hyperglobulinemia, macroglobulinemia, Waldenström liver cirrhosis in young girls.

PORPHYRIN METABOLISM

carcinoid syndrome, however, exemplifies how an observation of a patient who happened to be seen on a ward round can trigger off the imagination of an erudite clinician and bring to mind a multitude of memories and experiences and lead to the creation of a new syndrome. At that moment his brain must have acted as an electronic computer. This might also be called clinical intuition and it is commonly agreed upon that Jan Waldenström has a clinical imaginative eye. The importance of this new syndrome can hardly be overrated. It has opened our eyes to a hitherto unknown type of humoral activity of tumor tissue.

The portrait of Jan Waldenström would not be true without credit to his personal qualities, his alluring touch, his rapid apprehension and association, his intuition and empathy. These natural gifts are never so well displayed as when he is a little alarmed, as when faced with difficult problems, lecturing to a large audience or discussing controversial questions. One will also imagine that he uses his personal charm to fight scientific puzzles.

This volume pays tribute to Jan Wildenström, the versatile scientist, the sage physician, the learned teacher and medical philosopher. It marks a milestone, sixty years of life, thirty years of intense work in medical research. Friends, pupils, collaborators and admirers over the whole globe extend to him on this occasion their heartiest congratulations.

Sven Erik Björkman

I

PORPHYRIN METABOLISM

Porphyrin and Haem Biosynthesis and its Control

By C RIMINGTON

The essentially dynamic nature of the life of an entire organism and also of its constituent cells demands of necessity mechanisms for preserving homeostasis — a balanced order among the multitude of reactions taking place. This has been achieved at various levels and in various ways. The elaboration of organs in multicellular animals and a physiological division of labour between tissues such as muscle nerve cells gonads etc contribute towards such an end at a gross level of organization and the compartmentalization of intracellular reactions in various organelles carries the process on to the most intimate events of cellular metabolism. All this complexity demands highly efficient machinery for coordination.

The biosynthesis of the porphyrins and haems present in and essential to every cell offers no exception to the general rule: indeed it has been pointed out that the efficiency of this synthesis judged by the relative amounts of porphyrins and their precursors synthesised and excreted per day is of an astonishingly high order. The sys-

tem is nevertheless very adaptable and responsive: lowered oxygen tension rapidly induces a haemopoietin mediated polycythaemia and the haemoglobin output from the bone marrow of the dog may increase eight fold under the stress of chronic withdrawal of blood. In lower forms of life such as the crustacean *Daphnia* colonies may be observed to become red due to accumulation of haemoglobin under conditions of oxygen deprivation and to return equally rapidly to the non coloured state as a result of aeration (13). The induction or repression of cytochrome synthesis in yeasts (67) and bacteria (41) in response to changes in the oxygen content of the medium is another familiar example. Only in some pathological conditions do the regulatory mechanisms appear to break down and to result in gross accumulation and excretion of pigments of the tetrapyrrole series. Most of these diseases are genetically determined examples of "inborn errors of metabolism" but in both man and animals similar excesses of porphyrin production have been seen to follow

from poisoning by certain chemicals. Such "experimental porphyrias" have been closely studied in the hope of understanding more precisely the biochemical lesions responsible for the natural diseases. It is the object of this article to review some of these results and to determine how they illuminate the normal homeostasis of porphyrin and haem production.

The biosynthetic pathway through porphyrins to haem

Intensive study during the last decade has established the essential steps of the haem biosynthetic pathway (49, 50, 21). Certain enzymic steps still require clarification but it is accepted that the point of departure is the union of glycine and succinyl CoA under the influence of ALA synthetase and pyridoxal phosphate to form δ -aminolaevulinic acid (ALA), with α -amino β -ketoadipic acid as a probable short-lived intermediate. This event takes place within the mitochondria. ALA must then diffuse out into the cytoplasm where the next series of reactions takes place. ALA dehydratase condenses two molecules of ALA to form porphobilinogen (PBG), the first pyrrolic intermediate and it is to be noted that from this step onwards the pathway is irreversible so that the quantity of ALA undergoing pyrrolic condensation will determine the quantity of porphyrin which ultimately appears. Four molecules of porphobilinogen are cyclized with loss of 4 mols of ammonia to produce the first tetrapyrrolic substance, uroporphyrin

nogen III, a derivative of hexahydroporphyrin. The mechanism of this cyclization is not fully understood but two factors are necessary, an enzyme having porphobilinogen as its substrate and a co-factor, uroporphyrinogen isomerase, necessary for the production of the type III uroporphyrinogen, and which may be an integral part of the whole "porphobilinogenase" complex.

Stepwise partial decarboxylation of uroporphyrinogen III leads to coproporphyrinogen III; then for the next 2 steps coproporphyrinogen III must re-enter the mitochondrion. Here a "coproporphyrinogenase" brings about decarboxylation of the propionic acid side chains occupying the 2 and 4 positions and oxidation so that vinyl groups result, the product being protoporphyrinogen IX. These two steps have not been separated in *in vitro* systems and the course of the reaction is obscure. It is noteworthy that no substitute for oxygen as oxidant has so far been found for this *in vitro* system.

Insertion of ferrous iron into the tetrapyrrole to form haem is accomplished enzymatically within the mitochondria but for this to take place protoporphyrin must be present in the oxidized porphyrin state and not as the porphyrinogen; thus an oxidation step must intervene immediately prior to insertion of iron. The synthesis of haem by ferrochelatase (haem synthetase) preparations however requires iron in the ferrous form. Having once been formed within the mitochondria haem presumably diffuses into the

extra mitochondrial space where combination with specific proteins presumably takes place to produce the haemoprotein catalysts.

Importance of compartmentalization of reactions and of oxidation/reduction conditions

It will be evident from this brief summary of the biosynthetic pathway that discrete events demand particular localization and also particular oxidation/reduction conditions of the milieu. Alterations in the latter could affect the state of oxidation of thiol groups in the enzymes of the pathway many of which appear to be functional as SH proteins. A correct oxidation/reduction milieu may also be necessary not only for maintenance of the tetrapyrroles at the porphyrinogen level in which state they serve as substrates for many of the enzymes but also to satisfy permeability requirements of the various intracellular membranes.

This latter point raises another important consideration namely whether the intermediates along the pathway are present in the free state or are protein bound. If attached to a large protein molecule which may be necessary for the ensuing enzyme catalysed chemical transformation their velocity of migration will be considerably less than that of the free substance. Binding to a protein present in a particular intracellular compartment may also aid concentration within that compartment by preventing back diffusion. There is indeed

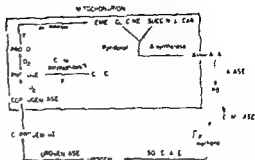


Fig. 1 Intracellular location of enzymes participating in haem biosynthesis [Reproduced by permission from Grinick and Levere (21)]

much evidence that some of the porphyrin intermediates serving as substrates for the biosynthetic enzymes do react while bound to proteins. Thus Porra and Falk (41) demonstrated covalent attachment of a porphyrin intermediate to protein during the enzymic transformation of coproporphyrinogen III to protoporphyrinogen IX. Similarly it appears to be a protein bound form of protoporphyrin which reacts with iron during the enzymic formation of haem (10, 40, 42). Iron itself is protein bound within the developing red cell (46, 11, 62). Granick and Levere (21) have summarized our knowledge concerning compartmentalization of the steps occurring in haem biosynthesis in the diagram reproduced as Fig. 1.

Limitation imposed by availability of substrates

It is perhaps obvious that the production of any substance through a biosynthetic pathway should be limited or subjected to control by the availability of the different materials re-

quired for its production. Thus iron-deficiency anaemia may result directly from an inadequate supply of iron to the body since iron is an essential constituent of haem and the accumulation of porphyrins by micro organisms cultured under conditions of iron deficiency is also a familiar phenomenon.

Porphyrin biosynthesis begins with the union of glycine and succinyl CoA, mediated by the enzyme ALA-synthetase with pyridoxal phosphate as one co factor and possibly ferrous iron as another (3). Deficiency of pyridoxine or pantothenate in the diet can cause an anaemia in animals and man which is corrected by supplying the vitamins (54, 66, 31). Glycine is so readily available in the diet and from intermediary metabolism that a deficiency of this amino acid is hardly ever likely to occur as a normal event. Lüs and Starr (68) have found, however, that a *Saccharomyces cerevisiae* mutant grown on minimal medium produced glycine so slowly that synthesis of haem was impaired and the organism contained only low levels of catalase and cytochrome c. These levels were raised to normal by addition of either glycine or protoporphyrin IX to the growth medium.

Succinyl CoA may be formed in several ways namely (1) from α ketoglutarate, derivable from the citric acid cycle, (2) by the direct reaction of succinate with coenzyme A catalysed by a nucleoside triphosphate. The latter appears to be either GTP or ITP in animal tissues whereas the enzyme from spinach requires ATP (3) from propionyl CoA (especially in ru-

minants) which combines with CO₂ in the presence of a carboxylating biotin containing enzyme to give methylmalonyl CoA. This latter is converted to succinyl CoA by a B₁₂ containing enzyme, (4) through the action of a CoA transferase on acetoacetyl CoA. In developing erythrocytes the most important pathway appears to be number 1 although number 2 is also operative. Thus Shemin and Kumin (56) blocked succinate oxidation in intact duck erythrocytes by the addition of malonate so that very little α ketoglutarate would be generated from the citric acid cycle. When methylene labelled succinate was now added to the system, incorporation of the label into haem took place to an extent suggesting that some 30—50 % of the required succinyl CoA could be derived directly from succinate. In line with this conclusion is the finding (3) that addition of ATP to haemolyzed chicken erythrocytes enhanced ALA synthesis only when succinate was the substrate. In the whole animal, inhibition of succinic dehydrogenase by administration of fairly large amounts of malonate has been shown recently (35) to increase the excretion of porphyrins and porphyrin precursors.

Pathway No. 1 is the route for succinyl CoA synthesis most likely to be affected by events in general intermediary metabolism. A closer inspection of this pathway in pig heart has revealed (38) that it includes several reaction sequences involving the lipothiamide system much as originally suggested by Rimington (47-48). These are set forth below.

(a) $\alpha \text{KG} + \text{Thiamine pyrophosphate (TPP)} \rightarrow \text{succinyl TPP} + \text{CO}$

(b) $\text{succinyl TPP} + \begin{array}{c} \text{S} \\ | \\ \text{S} \end{array} \text{ lipoyl enzyme} \rightleftharpoons \text{succinyl} \begin{array}{c} \text{S} \\ | \\ \text{HS} \end{array} \text{ lipoyl enzyme} + \text{TPP}$

(c) $\text{succinyl} \begin{array}{c} \text{S} \\ | \\ \text{HS} \end{array} \text{ lipoyl enzyme} + \text{CoA SH} \rightleftharpoons \text{succinyl S CoA} + \begin{array}{c} \text{HS} \\ | \\ \text{HS} \end{array} \text{ lipoyl enzyme}$

(d) $\begin{array}{c} \text{HS} \\ | \\ \text{HS} \end{array} \text{ lipoyl enzyme} + \begin{array}{c} \text{S} \\ | \\ \text{S} \end{array} \text{ FAD enzyme} \rightleftharpoons \begin{array}{c} \text{S} \\ | \\ \text{S} \end{array} \text{ lipoyl enzyme} + \begin{array}{c} \text{HS} \\ | \\ \text{HS} \end{array} \text{ FAD enzyme}$

(e) $\begin{array}{c} \text{HS} \\ | \\ \text{HS} \end{array} \text{ FAD enzyme} + \text{NAD}^+ \rightleftharpoons \begin{array}{c} \text{S} \\ | \\ \text{S} \end{array} \text{ FAD enzyme} + \text{NADH} + \text{H}^+$

The integrity of all these systems is essential for the biosynthetic supply of succinyl CoA to proceed smoothly. In particular it is necessary that oxygen be freely available for the operation of the cycle.

Effect of oxygen tension on biosynthesis of porphyrins

The effect of oxygen tension on the cytochrome and haemoglobin production of whole organisms has already been mentioned briefly and the fact that hormonal adjustments are often brought into play.

The tension of oxygen in the suspending medium also appears to have an important effect, however, on the individual enzymes participating in the haem biosynthetic pathway *in vitro*. Whilst such *in vitro* effects upon isolated systems are not necessarily a true indication of the state of affairs in the organized living cell they may nevertheless have some significance in helping us to understand the complexities of *in vivo* control. Falk et al (12) have used chick blood or washed erythrocytes with glycine as substrate for such experiments and have shown

that coproporphyrin production is maximal at about 1 % oxygen whilst the optimum for protoporphyrin and haem is in the region of 7 % oxygen, their formation being markedly depressed at higher oxygen tensions. Depression of protoporphyrin synthesis can be reversed by again lowering oxygen tension during the course of the incubation.

Control by end product inhibition

It has become apparent in recent years that the activity of several different biosynthetic pathways may be controlled by negative feed back inhibition, the end product of the pathway exerting an inhibitory action on the initial enzyme system. Such regulation is economical in that it prevents synthesis of the end product over and above the actual requirements of the cell or body as a whole.

Such phenomena have been studied mainly in bacteria but we do know from the early work of Whipple (64) that the bone marrow of the dog is capable of increasing its rate of synthesis of haemoglobin by some eight fold under conditions of severe anaemia.

ma Nevertheless, in the normal homeostatic state the balance sheet of pigment production and excretion by man shows an astonishing degree of efficiency, approximately 460 μ moles of haemoglobin being synthesised per day with a "waste" of only about 24 μ moles of porphyrin precursors and of less than 0.2 μ moles of porphyrins in the urine (33). Similarly the efficiency of the enzymic system which directs porphobilinogen to uroporphyrinogen III rather than the I series isomer has been calculated (21) to be of the order of 99.9 %.

That tetrapyrrole synthesis in bacteria is controlled by negative feedback inhibition of enzymes of the pathway is suggested by the accumulation of porphyrins occurring under conditions of iron deficiency and in the case of *Rhodospseudomonas spheroides* this phenomena has been studied in detail by Lascelles (30). The quantity of porphyrins formed without iron is about one hundred times greater than the amount of total tetrapyrroles (haems and bacteriochlorophyll) formed with iron. The excreted porphyrins therefore represent overproduction of an intermediate as a result of the failure of a control mechanism. The action of iron is also catalytic, some 100 μ m moles of coproporphyrin being prevented from appearing by only 2 μ m moles of iron citrate. Evidence that the actual inhibitor is a compound of iron viz haem, and that it acts upon the initial enzyme of the pathway follows from the observations that porphyrin accumulation by intact cells is inhibited by haem when

α ketoglutarate and glycine are the substrates, but not with ALA as substrate, and that the isolated ALA synthetase system is extremely sensitive to haemin, being inhibited significantly by 0.1 μ M of haemin. This inhibition is non competitive with any of the substrates or co factors of the synthetase. So far this negative feedback inhibition of ALA-synthetase by haem has only been established conclusively in the case of *R. spheroides* but it may well operate more widely (26). It may be mentioned at this point that, as pointed out by Lascelles (33) it is always coproporphyrin and not protoporphyrin which accumulates under conditions of iron deficiency, a fact suggesting that iron may play a part in the conversion of coproporphyrinogen to protoporphyrinogen. In the most recent work on this transformation Battle, Benson and Rimington (1) have observed that their highly purified enzyme developed a red colour on addition of α α' bipyridyl or phenanthroline or 8 hydroxyquinoline some degree of inhibition of the transformation was exerted by the first two compounds but none by 8 hydroxyquinoline sodium azide potassium cyanide or desferal in the concentrations used. Whether or not some peculiar form of iron linkage is present remains a matter for conjecture.

Control by end-product repression

Apart from the possibility of a degree of control by end product inhibition through the operation of a negative feedback in which the end product

inhibits the initial enzyme involved in its formation biosynthetic systems appear to be regulated mainly by a more complex machinery involving the genetic material of the cell. This is termed control by end product repression and our knowledge of it has been derived almost entirely from bacteria. The theory originated by Jacob and Monod, (27-28) may be stated in its simplest form with definition of terms as follows: a structural gene a segment of chromosomal DNA carries the information for a particular enzymatic protein. This it imparts to a short lived type of RNA messenger RNA which in turn carries the information to the ribosomes where synthesis of the protein takes place. Synthesis of messenger RNA is initiated by another region of DNA designated the operator gene. Structural gene and operator gene together make up an operon.

Should there be no restraint upon this system continuous uncontrolled production of messenger RNA and of the specific protein would result. This is avoided by the action of yet another segment of DNA a regulator gene producing a repressor which has the ability to block reversibly the activity of its operator gene. The repressor in turn may be rendered inactive by combination reversibly with an effector. Conversely an effector may enter into combination with an aporepressor the combination having repressor activity. The effectors would appear to be relatively small molecules less complex than proteins. Normally therefore biosynthesis of an enzyme

protein like ALA synthetase is held firmly in control by its regulator gene and repressor. Products of one biosynthetic chain may act as effectors in other synthetic processes, so co-ordinating different pathways. Effector activity may also be a property of some hormones e.g. oestrogen which are able to stimulate protein synthesis *in vivo* (24).

The theory which in its fully developed form is very complex is nevertheless able to explain such phenomena as enzyme induction in bacteria and the segregations observed and predictable from bacterial genetics. Its main points have been recently summarized by Brenner (2). The properties required of repressors are just those provided by allosteric proteins binding of an inducer molecule at one site being able to reduce the affinity of another site for the operator. This may be achieved by deformation charge alteration or otherwise.

Formation of ALA synthetase in growing cultures of *R. spheroides* is repressed by addition of haemin in concentrations as low as 0.01 mM²² and this repression appears to be specific since other porphyrins or metal complexes are devoid of activity. It is of interest that haemin also exerts repression upon ALA dehydratase the next enzyme in the biosynthetic sequence this being an example of co-ordinate repression a phenomenon often witnessed in bacterial biosynthetic systems.

In the higher organism there is strong evidence that the synthesis of the haemoprotein haemoglobin is not

Nevertheless, in the normal homeostatic state the balance sheet of pigment production and excretion by man shows an astonishing degree of efficiency, approximately 460 μ moles of haemoglobin being synthesised per day with a "wastage" of only about 24 μ moles of porphyrin precursors and of less than 0.2 μ moles of porphyrins in the urine (33). Similarly the efficiency of the enzymic system which directs porphobilinogen to uroporphyrinogen III rather than the I series isomer has been calculated (21) to be of the order of 99.9 %.

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Apart from the possibility of a degree of control by end product inhibition through the operation of a negative feedback in which the end product

the hypothesis that haem is required for globin synthesis

Increasing concentrations of hemo globin in the normoblast nucleus act like histone to combine with, condense and inactivate DNA (step 5). Globin synthesis can now continue only on preformed m RNA and stops when this m RNA is depleted. This DNA inhibition by hemoglobin will also prevent the formation of new ALA synthetase. However, even with cessation of the synthesis of this enzyme haem formation could continue for many hours after globin production has stopped by utilization of the preformed ALA synthetase. This would lead to an overproduction of haem and porphyrins. Since, under normal conditions this overproduction does not occur there must be an additional control mechanism to regulate the synthesis of ALA. It is postulated in step 6 that the free haem appearing after the termination of globin synthesis acts directly to inhibit ALA synthetase and prevent an overproduction of porphyrins" as is the case in *R. Spheroides* systems. This ingenious scheme provides a background against which to discuss recent observations upon experimental porphyrias.

Experimental porphyrias

The first experimental production of a porphyric state was accomplished by Stokes (27) in 1893 by the administration of sulphonal to rabbits and dogs. A number of other substances were subsequently shown to influence

porphyrin metabolism but it is only since Schmid and Schwartz (23) demonstrated in 1952 that Sedormid administration caused in hepatic form of porphyria in rabbits that extensive study has been devoted to these phenomena. There is now a relatively long and well documented list of chemical substances which are capable of producing an experimental porphyria. Among these may be mentioned the sedormid type drugs (18), certain barbiturates (17), griseofulvin (9), 3,5-diethoxycarbonyl 1,4-dihydro-2,4,6-trimethyl pyridine (DDC) (7, 23, 22), 2-allyloxy-3-methylbenzamide (15), hexachlorobenzene (13) and other chlorinated benzenes (21). A good deal of discussion took place as to whether the excessive amounts of porphyrins formed in the excreta were due to overproduction or to under utilization on account of some metabolic block. This latter view seemed supported by the fact that the different porphyrinogenic agents did not all produce the same pattern of porphyrin excretion; however other considerations especially those of a quantitative nature, pointed to overproduction as the true explanation (18). Thus for example allylisopropylacetamide induced porphyria is not accompanied in rabbits by anaemia in spite of a daily loss of about one half the quantity of porphyrin which is synthesised daily for haemoglobin production (18). Merchante Wajchenberg and Schwartz (39) showed that liver homogenates from sedormid treated rats converted porphobilinogen to porphyrin at the same rate as did control homogenates.

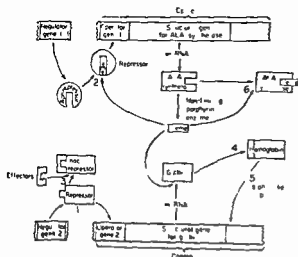


Fig 2 Hypothetical scheme for control of haemoglobin synthesis (Reproduced by permission from Granick and Lere (21))

mally subjected to controls similar to those outlined above and operative in micro organisms. Thus the production of haem and of globin in erythropoietic cells seems to be regulated so that under normal conditions they arise in the required 1:1 ratio. Granick and Lere (21) have constructed a hypothetical scheme embodying controls in haemoglobin synthesis which could explain many of the known facts (Fig 2). It assumes that globin genes are turned on independently of haem synthesis but that because of globin synthesis the rate of haem synthesis is increased and that no globin polypeptide is completed in the absence of haem. The steps in the scheme are explained as follows.

Prior to the initiation of haemoglobin synthesis the operon responsible for globin synthesis is activated but kept repressed by its corresponding regulator gene as in step 1. The operon concerned with the synthesis of ALA

synthetase, the limiting enzyme in the haem biosynthetic chain, is also repressed. However, here it is assumed that the aporepressor produced by regulator gene 1 requires haem for effectively repressing the operon for ALA-synthetase (step 2). With the entrance of specific effector substances into the cell the repressor of the globin operon is rendered inactive (step 3), allowing globin synthesis to commence. Erythropoietin or possibly metabolites from neighbouring cells are substances which might act in this manner to initiate globin synthesis. The globin thus formed now utilizes any available free haem for hemoglobin formation (step 4) depriving the repressor of the ALA synthetase operon of its effectiveness and allowing the synthesis of this enzyme to proceed at a maximal rate. Since the other enzymes in the haem biosynthetic chain are already present in non-limiting amounts haemoglobin production is at this point limited only by the availability of substrates.

To explain the 1:1 ratio of the haem to globin monomer formed by the red cells it is postulated that the globin will not come off the polysome unless it has been correctly folded around the haem. Rich has suggested that when two adjacent monomers are completed they will come off the polysome together as a dimer. Hammel and Bessman (25) found that haem stimulates globin synthesis when added to a preparation of hemolyzed avian erythrocytes which consisted mostly of nuclei. This supports

sumably haem formation proceeded readily on addition of ALA to cultures at a time when addition of succinate and glycine was without effect. By passing ALA synthetase and providing intracellular haem in this way was found to stimulate globin synthesis and haemoglobin production. The level at which this effect is exerted appears to be at the ribosomal synthesis of globin since it was sensitive to puromycin but not to actinomycin D. Will (60) has been led to similar conclusions.

Concomitant metabolic disturbances caused by porphyrinogenic drugs

As early as 1905 Schwartz (35) observed a disturbance of lipid metabolism in experimental porphyria and an increased rate of fatty acid synthesis by the livers of such rats was demonstrated by Labbe Hanawa and Lottsfeldt (29) and raised serum cholesterol, total lipids and phospholipids by Taddei Nordstrom and Watson (18). Reviewing records of all cases of hepatic porphyria the latter authors (18) found hypercholesterolemia to be of common although inconstant occurrence in acute intermittent and mixed porphyria. Increased ascorbic acid excretion in the rat with experimental porphyria has also been observed by Ginsburg and Dowdle (16) and De Matteis (6). Such metabolic disturbances are by no means characteristic of the experimental porphyric state; in fact they are frequent accompaniments of the administration of a variety of different

drugs. They do, however, suggest stimulation of enzyme synthesis in the liver and the increases in ascorbic acid excretion and in hepatic UDPG dehydrogenase activity which follow in connection with chlorotone or barbital are both preventable by ethionine. In the case of the porphyrinogenic drug ALA De Matteis (6) found that the increased excretion of ascorbic acid was not significantly affected by administration of glucose, actinomycin D or ethionine whereas each of these substances inhibits the porphyria in rats. It would appear that there is no close connection between porphyria on the one hand and increased lipid and ascorbic acid synthesis on the other.

Human Acute Intermittent Porphyria

In any attempt to relate findings from drug induced experimental porphyrias in animals to the diseases occurring in man one is met at the outset with the difficulty that very little is known about the enzyme levels in the tissues of human porphyrics. This is largely due to the difficulty of obtaining sufficient liver and bone marrow material for adequate quantitative studies. Only in one case of acute intermittent porphyria is there definite evidence of a raised level (between 7 and 14 fold) of hepatic ALA synthetase (19). Other enzymes of the porphyrin biosynthetic pathway were present in normal amount with the exception of ALA dehydratase which was possibly slightly increased. These important findings are strongly suggestive of an

On the other hand Gibson (15) noted that the liver and kidney, but not bone marrow or spleen, of sedormid intoxicated rabbits showed a marked rise in ALA-dehydratase activity. Granick and Ulat (22) then made the important observation that the concentration of ALA synthetase in guinea pig liver was increased forty-fold above its normally low level by dosing the animals with DDC. Other enzymes of the biosynthetic chain were not appreciably affected, they are normally fairly active in liver. These findings indicated that the rate of hepatic porphyrin synthesis is limited primarily by the activity of ALA synthetase.

From the discussion set out in the earlier part of this paper it will be clear that an increase in activity of a tissue enzyme could result from either activation of temporarily inhibited enzyme or from a *de novo* synthesis of the enzyme by the tissue cells. That the porphyrinogenic drug did in fact induce new synthesis of enzyme followed from the finding (19) that the same porphyrin increase could be observed by adding AIA to chick embryo liver cells growing in tissue culture but that the effect was blocked by also adding inhibitors of protein synthesis like actinomycin D. By this elegant technique two new substances, chlorotone and aminopyrine, were added along with AIA, DDC, Griseofulvin, hexachlorobenzene, sulphonal and Dial to the list of porphyrinogenic agents (19). A still more extensive list which includes many well known therapeutic agents, is given by Granick

(20), they are all contra indicated for subjects known to have porphyria.

It is of interest that in this tissue culture system the addition of haem to the medium did not repress the induction of porphyrin synthesis by AIA (19), whereas it will be remembered that it does repress ALA synthetase formation in *R. Spheroides* (32). The explanation of the difference may, however, lie in a possible impermeability of the liver cells to haem.

Assuming that haem is necessary for repression in this system also, then reference to Fig. 2 will indicate that the de-repression of the operator gene responsible for ALA-synthetase production could be brought about by either (1) combination of the inducing (de-repressing) agent with haem itself or (2) combination with the *apo* repressor so as to block its union with haem. In either case, the net result would be prevention of formation of the *apo* repressor/haem complex. At the present time it is impossible to distinguish between these alternative possibilities but the way is clearly open for chemical investigation. Despite wide differences in overall chemical structure among the known porphyrinogenic drugs work within particular series has already succeeded in defining the features essential for activity within such a series (18-37). In later experiments Levere and Granick (34) have used the chick blastoderm to study haem and globin synthesis *in vitro*. ALA synthetase was again found to be the limiting factor for haem synthesis by these erythropoietic cells since porphyrin and pre-

sumably haem formation proceeded readily on addition of ALA to cultures at a time when addition of succinate and glycine was without effect. By passing ALA synthetase and providing intracellular haem in this way was found to stimulate globin synthesis and haemoglobin production. The level at which this effect is exerted appears to be at the ribosomal synthesis of globin since it was sensitive to puromycin but not to actinomycin D. Wilt (6a) has been led to similar conclusions.

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(20), they are all contra indicated for subjects known to have porphyria.

It is of interest that in this tissue culture system the addition of haem to the medium did not repress the induction of porphyrin synthesis by AIA (19), whereas it will be remembered that it does repress ALA synthetase formation in *R. Spheroides* (32). The explanation of the difference may, however, lie in a possible impermeability of the liver cells to haem.

Assuming that haem is necessary for repression in this system also, then reference to Fig. 2 will indicate that the de repression of the operator gene responsible for ALA synthetase production could be brought about by either (1) combination of the inducing (de repressing) agent with haem itself or (2) combination with the apo repressor so as to block its union with haem. In either case the net result would be prevention of formation of the apo repressor/haem complex. At the present time it is impossible to distinguish between these alternative possibilities but the way is clearly open for chemical investigation. Despite wide differences in overall chemical structure among the known porphyrinogenic drugs work within particular series has already succeeded in defining the features essential for activity within such a series (18-37). In later experiments Levere and Granick (34) have used the chick blastoderm to study haem and globin synthesis *in vitro*. ALA synthetase was again found to be the limiting factor for haem synthesis by these erythropoietic cells since porphyrin and pre-

protein nature and may be an enzyme which deviates succinic acid into pathways other than that of porphyrin biosynthesis

It will thus be clear that although the concept of a genetic abnormality resulting in a failure of repression of ALA synthetase in acute intermittent porphyria is attractive there remain many problems to be solved before this hypothesis can be fully accepted. In this connection a profitable line of study might be the excessive catalase production observed in a *R. Sphaeroides* mutant by Clayton and Smith (2). This organism would appear to present another example of a genetic defect resulting in failure to control the synthesis of a particular enzyme catalase could comprise from 5 to 20 per cent of the total dry weight of the mutant.

Addendum

A recent report (28a) states that induction of a succinyl CoA isoenzyme occurs prior to that of ALA synthetase in livers of mice receiving DDC. Tri-carboxylic acid cycle turnover is unchanged but succinate and succinyl CoA pools increase. There may be a connection between these important findings and that of Gajdos Török (14).

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induction or de-repression of ALA synthetase but the mechanism by which it could be brought about is not known. Acute intermittent porphyria is a dominantly inherited disorder and Tschudy *et al* (59) point out that any defect of a regulator gene, that is a constitutive regulator mutation would be expected to be have as a recessive. A constitutive operator mutation in acute intermittent porphyria is discussed by Watson *et al* (61) for congenital erythropoietic porphyria could explain the genetic and biochemical findings but one would have to accept the over production of porphyrin precursors as responsible for the neurological symptoms, which is contrary to experience. Alternatively there may be a single genetic defect which lies outside of the h_um biosynthetic pathway but which leads secondarily to induction of ALA synthetase.

End product repression might be defective if, for example, haem were involved in repression of hepatic ALA synthetase as it appears to be in micro organisms, but was not produced at the normal level owing to a partial block in the biosynthetic pathway. There is, however, no evidence for such a block. Another possibility which should be considered is that in acute intermittent porphyria there is a defect in an *apo* repressor molecule which normally only becomes effective as a repressor of the operator gene for ALA synthetase when it is combined with a product such as haem. Inability so to combine would

deprive the system of control by this mechanism.

Support for the idea that there is an induction of ALA synthetase in acute intermittent porphyria (59) is afforded by the effect of glucose. Rose, Hellman and Tschudy (52) first observed that the degree of experimental porphyria produced in animals by administration of a porphyrinogenic drug was influenced by the diet, a high carbohydrate or protein diet largely suppressing the porphyric disturbance. Later this observation was extended to the human patient with acute intermittent porphyria (63). The induction of hepatic ALA synthetase by ALA was found to be inhibited by glucose (60).

The ability of carbohydrate to inhibit the induction of certain enzymes has been observed in the past mainly in bacteria. The phenomenon is called the 'glucose effect' or 'catabolite repression'. It has been suggested that glucose sensitive enzymes are capable of converting their substrates into metabolites which the cell can also obtain independently and more readily from glucose whereas this is not so in the case of glucose insensitive enzymes. The difficulty about applying this concept to the induction of ALA synthetase is that no route to porphyrins from glucose has been demonstrated. In this connection the demonstration of the biosynthesis by *R. Sphaeroides* of an inhibitor of porphyrinogenesis which is accelerated by ATP may prove to be of much significance. According to Gydos and Gydos-Török (14) the inhibitor is of

Some recent advances in the problem of erythropoietic porphyria

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The concept that porphyria congenita Gunther is basically related to erythropoiesis and might best be designated as porphyria erythropoietica in contradistinction to the much larger group of hepatic porphyrias was first presented in 1951 (1). It was based on the observation that the bone marrow in congenital porphyria contains large numbers of fluorescing normoblasts (2, 3) and the circulating erythrocytes invariably contain excessive uro- and coproporphyrin in contrast to hepatic porphyria in which the liver rather than the developing red cells is rich in porphyrins or porphyrin precursors.

There is also the interesting fact that when excessive hemolysis was eliminated by means of splenectomy in congenital porphyria the amount of uroporphyrin in the circulating blood and excreta declined sharply. These findings have been confirmed both in additional cases of the human disease and in the highly similar bovine form (4). Our group in collaboration with the Veterinary School of Medicine has had available for several years a small herd of these animals including both those homozygous for the trait with pink teeth and marked photosensitivity and heterozygotes without any discoverable abnormalities either clinical or biochemical. The erythropoietic nature of the bovine disease is similarly apparent in respect to the many fluorescing normoblasts in the bone marrow and the striking effect of the stimulus provided by bleeding. This is followed promptly by an outspoken increase of the circulating erythrocyte porphyrins—especially uroporphyrin and concomitantly the urinary uro-

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Some recent advances in the problem of erythropoietic porphyria

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2) by differential centrifugation Uro porphyrin rich cells in the bovine porphyrin are more fragile to hypotonic saline (4) and like the reticulocytes are in the lesser density fraction. We do not have fractional osmotic fragility data for human porphyrin cells but there is strong reason to believe that they would behave in a manner opposite to that of the bovine cells. In a separate study with Johnson and Schwartz (9) it has been shown that young bovine cells normal as well as porphyrin are relatively more fragile while those of the human and dog are in the more resistant fraction in accord with previous observations (10).

In terms of the distinction of porphyrocytes or uroporphyrin rich cells no significant difference was found in the proportion of type 1 in the new uroporphyrin formed on incubation of PBG in hemolysates representing the more and less fragile fractions despite the fact that there was a striking difference in the native uroporphyrin content per gram of hemoglobin in these fractions. A much more extensive comparison has been made of hemolysates from lesser and greater density fractions as with this method it is possible to note the degree of correlation with the percentage of reticulocytes. This work is to be described elsewhere and it will suffice to note here that the differences have not been consistent nor of sufficient magnitude to suggest the presence of two distinct cell types in other words porphyrocytes representing the genetic abnormality vs normal erythrocytes (11).

B The origin of the free porphyrins of excreta, bone and other tissues

It has been pointed out in the past that one cannot explain the very great excess of free porphyrin in the excreta on the basis of destruction of circulating red cells even if one assumes a marked shortening of the red cell lifespan. Illustrative data are presented in Table I.

Table I *Erythropoietic porphyria*
Uro and coproporphyrin of excreta
us erythrocyte porphyrins

Case MM Q 2a

ca 200 mg urinary + fecal porphyrin/d

Total circulating erythrocyte porphyrin/d

ca 10 mg based on a 24h r.b.c life span

$Cr^{4+} T 1/2$ 1a & d

Thus only 0.5—0.7 mg/d calculated for
20—30 d life span

On the basis of the circulating red cell mass and the erythrocyte porphyrin concentration it may be calculated that a complete turnover every few minutes would be required to begin to account for the large amounts of uro and coproporphyrin excreted without considering storage in bone and other tissues. For a time we considered the possibility that there was excretion from the porphyrin rich normoblasts in the bone marrow and in the broad sense this seems essential. I believe now however that this is a passive excretion related to extrusion of the porphyrin rich nuclei. Fluorescence microscopy makes it fully evident that nuclei contain much more porphyrin than the cytoplasm and the fluorescence spectrum is entirely that of uroporphyrin. Fig 1 shows the fluo

porphyrin increases. As will be emphasized in the following, there is also a remarkable increase in the percentage of fluorescing normoblasts in the bone marrow.

Are the normoblasts uni- or bimodal in respect to the genetic error?

The fluorescing normoblasts in the bone marrow, both human and bovine, often exhibit nuclear inclusions which contain heme (2, 3). Whether this is actually in the form of hemoglobin has not yet been determined. The nuclei also contain a large amount of uroporphyrin, obviously much more than in the cytoplasm of the normoblast. At the time of the first recognition of these peculiarities, the possibility was suggested that there are two varieties of normoblasts in this disease, one representing the genetic error which might be termed a "porphyroblast", the other normal (2, 3). Recent histologic studies (5, 6) appear to support a bimodal concept of this type; however, we have obtained important evidence more in favor of a single modality.

1. In a study with W. Runge (7, 8) it was found that when bovine porphyrics are bled repeatedly over a period of a week or ten days sufficient to produce moderate anemia and stimulus to the bone marrow, the percentage of fluorescing normoblasts increased in striking fashion from a baseline value of 40—50 to a range in the various experiments of 82—92%. Comparable bleeding in normal animals is attended by only a very slight

increase from a resting value of 1—2% to 2—4%, also the rapid disappearance of fluorescence in these normal cells on exposure to UV light indicates that the porphyrin is mainly proto rather than uroporphyrin as in the porphyric cows. This finding is at least very difficult to reconcile with a bimodal concept, unless one were to assume that only "porphyroblasts" increase in response to bleeding, in contrast to normal erythroblasts. This, however, would be contrary to the general evidence that bleeding induces a marked increase of normal erythroblasts in any and all species in which this has been studied.

2. It has been held that only genetically abnormal "porphyroblasts" give rise to the porphyrocytes of circulating blood, these being the cells which contain the uroporphyrin. Larizza (5) believes that the porphyrocytes are faulty cells of short life span, fundamentally related to the increased hemolysis which is often exhibited in these cases and may be outspoken. Since the genetic enzymatic abnormality whatever it may be (and this is discussed again in the following), must include an overproduction of type I uroporphyrin, it is reasonable to assume that if the porphyrocytes could be suitably concentrated, the hemolysis of such a concentrate would be expected to exhibit a much greater formation of uroporphyrin I than that of concentrates from cells of low uroporphyrin content from the same sample of blood. There are two ways in which separation of this type can be achieved: 1) by differential osmotic lysis

blast nuclei on extrusion must provide a very considerable proportion of the large amounts of porphyrin which are either excreted or stored. There is an analogy in the hyperuricemia of states associated with increased nuclear turn over such as leukemia especially after radiation and polycythemia. The uricosuria is marked and, of course there is often deposition in the skeleton even with manifest gout.

C *The affinity of nuclear material for uroporphyrin*

Studies with W. Runge and J. Yarbrow have made clear that uroporphyrin readily enters the nuclei of living cells such as those of rat or bovine liver or Ehrlich ascites tumor cells. The fluorescence of the porphyrin is largely or completely masked first becoming apparent on treatment with dilute HCl. This was well demonstrated in the chromosomes of Ehrlich ascites tumor cells undergoing mitosis. We have recently obtained evidence that nucleohistone in contrast with either DNA or histone forms some type of complex with uroporphyrin. This study is still in progress and will be described in detail elsewhere. The finding that uroporphyrin enters nuclei raises the question is yet unanswered whether the normoblast nuclear uroporphyrin is formed in the cytoplasm or nucleus or both.

D *The problem of detection of the heterozygous state*

It is well known that classical erythropoietic porphyria or morbus Gunther is

transmitted as an autosomal recessive trait. We have been unable to detect the heterozygous condition by means of porphyrin analysis either in the human or bovine disease known bovine heterozygotes, as determined by the breeding record, have failed to reveal differences from the normal. In one family in which two siblings were homozygous with characteristic phenotype, the mother and two other siblings were entirely normal both clinically and by porphyrin analyses. The father died of heart disease without having had any manifestations of porphyria. Heilmeyer and co-workers have reported significant increases of erythrocyte uroporphyrin in asymptomatic relatives of a human porphyric and they believe that these results have permitted detection of the heterozygous condition (13).

E *In "overproduction" disease with out evidence of a lack of isomerase*

It has often been stated that the basic genetic abnormality in this disease is a lack of isomerase. Nevertheless it has been known since Fiescher's classical studies in the case of Petry that while the urine and fecal porphyrins corresponded in configuration with aetioporphyria 1 the protoporphyrin of the hemoglobin of circulating red cells corresponded with aetio series III (16). This in fact, first delineated the dualism of the porphyrins in nature subsequently shown to have much wider implications in biology. If there were a deficiency of isomerase as a structural genetic error

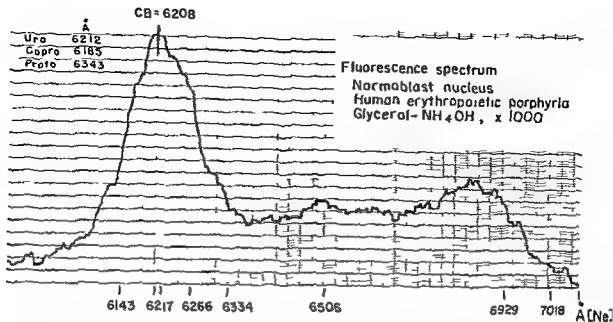


Fig 1 Fluorescence spectrum of the nucleus of a single intact normoblast from the bone marrow biopsy of a human case of erythropoietic porphyria recorded by W Runge with the microfluorospictrphotometer (12). Stained preparation treated with glycerol NH₄OH 3:1 linear magnification 1000. CB=center band Ref values from Dhéré and Bois (34)

rescence spectrum of a single normoblast nucleus as recorded with the microfluorophotospectrometer a unique apparatus which has been constructed over the past 17 years in our laboratory and is described in detail elsewhere (12). The spectral distribution curve in Fig 1 is mainly that of uroporphyrin. This requires some explanation of the fact that approximately equal amounts of copro- and uroporphyrin are found in the excreta: the majority of the former in the feces and of the latter in the urine in accordance with Fischer's initial discovery and nomenclature (13, 14). The most reasonable explanation is that uroporphyrin (ogen) liberated from the normoblasts is converted to coproporphyrin either in the bone marrow or in the liver to be excreted mainly in the

bile. There is no reason to doubt that uroporphyrinogen may be converted in part to coproporphyrinogen either in the cytoplasm of normoblasts or in non nucleated cells especially reticulo cytes. Excessive coproporphyrin in the circulating red cells especially the lesser density fraction is readily demonstrable. There is also the question whether uro- or coproporphyrin can be secreted by or in some manner lost to the plasma from intact erythrocytes. We have not been able to detect any loss from freshly collected bovine porphyric red cells when kept in motion in plasma incubated over a number of hours. Thus it is recognized does not exclude the possibility that there might be secretion *in vivo*. All things considered however it seems clear that the uroporphyrin laden normo-

the enzyme incubated *in vitro* with coproporphyrinogen I there was no evidence of formation of protoporphyrin. It is nevertheless conceivable that the enzyme might partially lose its specificity under certain circumstances *in vivo*. While these observations are of considerable interest, the amounts of the differing mesoporphyrins and the frequency of their occurrence was too small to indicate a fundamental significance for the disease and its genetic abnormality.

In respect to the concept of an isomerase deficiency it is important to emphasize that there is actually a considerable overproduction of type III porphyrin, including protoporphyrin, in this disease. For one thing, the free porphyrins of the excreta, although mainly type I, include a distinct proportion of type III, ranging from 5 to 20% in various determinations. This represents a much larger amount than is found in the normal excreta. More important, however, is the fact that there is generally a considerable excess of urobilinogen in the feces. This has been shown 1) not to differ from the stercobilin of normal feces (20) and 2) to consist in large proportion of the early labelled fraction (20, 21, 22). The general significance of the early labelled bile pigment has been considered in a recent review (23) and need not be dealt with in any detail at present. It is often related in considerable measure to ineffective erythropoiesis of which there is essentially no evidence in erythropoietic porphyria. The bone marrow in this disease is usually hyperplastic and normoblastic

and there is good reason to believe that the heightened erythropoiesis is a response to increased red cell destruction, at least in part by the spleen. However, a marked increase in early labelling has been noted even when there was no evidence of increased hemolysis (20, 22). It is evident that the extrusion of the normoblast nuclei containing heme inclusions, as referred to in the foregoing, provides a ready source of heme for conversion to bile pigment either in the bone marrow or after transport to the liver. This may not be the only source of early labelling in this disease but it is reasonable to believe that it is a highly important one. From the standpoint of the question of isomerase deficiency, these findings indicate that there is actually an overproduction of heme and consequently of protoheme 9 and the corresponding bile pigment.

If the amounts of type III porphyrin and urobilinogen of the excreta are compared with those of the type I porphyrins and with average normal data, it is quite evident that there is a highly significant overproduction of type III as well as type I, although the amount of the latter is relatively much greater than in the normal individual and this of course is basic to the phenotype of the disease. Such a comparison is shown in Fig. 2 in which the essential data from one of our cases of erythropoietic porphyria is contrasted with average normal data.

Erythropoietic porphyria is thus to be regarded as a disease of overproduction rather than actual enzyme deficiency or block. The overproduction

one would anticipate a striking diminution in heme and hemoglobin synthesis with a hypochromic anemia except if there were compensatory excessive formation of type I proto heme or if the isomerase deficiency were limited to a relatively small colony of abnormal cells. Fischer could not detect evidence of the first of these possibilities showing that the protoporphyrin of Petry hemoglobin was type 9 corresponding with the actio III series. This depended on conversion of proto to mesoporphyrin. Fischer commented on the fact that the mother liquor was dark, hence the possibility of a small proportion of type I isomer could not be excluded (16). My associates and I have reexamined this question in considerable detail (17). In the earlier course of our investigations a number of years ago prior to the availability of chromatographic techniques now available, the sodium salt solubility (18) and melting points of porphyrin methyl esters obtained after hydriodic acid reduction of hemin suggested the presence of a type I isomer. Later, however, it became clear that the hydriodic acid method yields other unidentified porphyrins in addition to mesoporphyrin. In all of our more recent studies protoporphyrin has been prepared from hemoglobin powder by the method of Grinstein (18). The total protoporphyrin thus obtained has then been converted to mesoporphyrin by catalytic hydrogenation with palladium following which a sample of the entire mesoporphyrin has been subjected to paper chromatography (7, 17). A sample of crystalline hemin

from the case Petry was very kindly provided by Prof. Alfred Treibs Technische Hochschule, Munchen. After conversion to proto, thence to mesoporphyrin, only type 9 (III) was demonstrable by paper chromatography. Many samples of bovine and human porphyrin hemoglobins, both from circulating red cells and from those of the spleen, have thus been examined in search for an isomeric protoporphyrin. In all but three instances there was no suggestion of any porphyrin but type 9 in the meso fraction. The three exceptions included the spleen from a newborn porphyrin calf, the spleen from a case of human porphyria¹ and one sample of bovine porphyrin red cells. A distinct spot was observed on the paper chromatogram of each of these having the same R_f as mesoporphyrin 1. The human spleen yielded a sufficient amount of a differing mesoporphyrin in the 0.8% NaOH soluble fraction to permit crystallization. This comprised but a small proportion of the total mesoporphyrin most of which was in the 0.8% NaOH insoluble fraction. The crystalline methyl ester from the soluble fraction exhibited the dimorphic melting point of mesoporphyrin 1 in addition to the paper chromatographic behavior of this isomer. The rare occurrence of small proportions of type I isomer as represented in these three exceptions is not understood. Sano and Grinnick (19) have shown that the coproporphyrinogenase responsible for conversion of coproporphyrinogen III to protoporphyrinogen III (III) is highly specific for type III. With a relatively pure sample of

(porphobilinogen) In acute porphyria much of the excess of ALA and PBG is promptly excreted by the liver cells and appears in the urine. In erythropoietic porphyria an increased amount of these precursors formed in the normoblasts is at once converted to uroporphyrinogen, the type III moiety of which is then largely converted to proto heme while the type I porphyrins are disposed of as already discussed. Excesses of PBG and ALA in the urine in erythropoietic porphyria have not been observed and since an excessive formation of these substances may be taken for granted the lack of increase in the urine is best explained on the basis of retention in the developing red cells and rapid conversion to porphyrin.

† Other forms of erythropoietic porphyria

In any discussion of classical erythropoietic porphyria attention must also be given to the remarkably different protoporphyria first described by Magnus and Rimington (26) and which has been studied so extensively and to such good purpose in Professor W. Aldenstrom's clinic especially by Dr Birgitta Hager-Aronsen. Reference may be made to her excellent survey of various aspects of this curious disease in the recent literature (27). There is little doubt that many cases have been classified as solar erythema, urticaria or eczema in the past without recognition that they were suffering from porphyria partly because of the usual absence of a vesiculo bullous

eruption (hydra), but more important, because reliance was placed on urinary porphyrin analyses and because protoporphyrin is not excreted in the urine. It is now fully established that the most constant feature of the disease is the remarkably heightened erythrocyte protoporphyrin concentration. Our experience with a number of cases of this well defined form of porphyria has been described in a recent paper (28).

Heilmeyer and Clotten (29) have described what they regard as an independent form of erythropoietic porphyria i.e. "coproporphyria". It is highly interesting that their case was the same as that of Kosenow and Treibs (30) published 12 years earlier under the title of "Lichtüberempfindlichkeit und Porphyrinämie". They noted many fluorescing erythrocytes but were unable to identify the erythrocyte porphyrin with certainty as protoporphyrin. They found however that the plasma contained a porphyrin metal complex which they stated was not that of protoporphyrin on spectroscopic grounds. Nevertheless the feces contained a large amount of protoporphyrin and very little coproporphyrin and this of course is quite in accord with protoporphyria. There was no increase of urinary porphyrin. With these observations in mind the findings of Heilmeyer and Clotten 12 years later are of great interest. The urine was again entirely normal. The erythrocytes contained large amounts of coproporphyrin entirely type III isomer, a moderate increase of proto and a relatively marked increase of uro

Urobilinogen and Porphyrin Excretion Related to Proportions Formed as Isomers of Series I or III

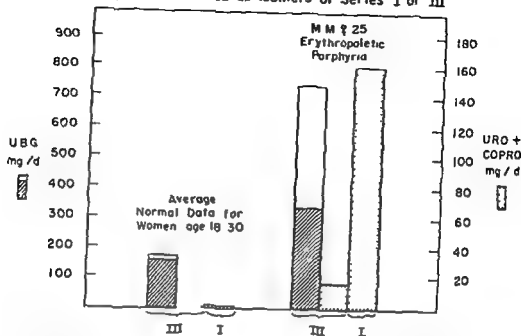


Fig 2 Amounts of porphyrin isomers I and III and urobilinogen 9a (III) in the excreta in erythropoietic porphyria as contrasted with average normal values for same sex and age range. The upper open areas in the urobilinogen (UBG) columns simply indicate that a much larger proportion is of the "early labelled" type in erythropoietic porphyria than in the normal. This was calculated in terms of total circulating hemoglobin and $\text{Cr}^{51}\text{T } 1/2$ (Table I).

is characterized, however, by an imbalance in which the deaminase activity is relatively much greater than if the normal ratio had been maintained. Taking into account the recessive character of the disease a porphyrin overproduction such as this represents is satisfactorily explained as a constitutive regulator mutation with induction of δ -aminolevulinic acid (ALA) synthetase, in the developing red cells. Various possibilities based on such a mutation have been discussed in detail in a separate communication (7). Granick (24) has shown quite clearly that porphyrogenic chemicals act by de-repression and consequent induction

of ALA synthetase in liver cells and Tschudy (25) has recently demonstrated a highly significant increase of ALA synthetase in the liver of a fatal case of human acute porphyria. The striking phenotypic differences between acute porphyria and erythropoietic porphyria assuming that ALA synthetase induction is common to both, might well be explained on the basis of differences between liver cells and normoblasts, both in terms of permeability to ALA and PBG and to relative rates of utilization of these compounds as a function of the concentration of enzymes in the porphyrin biosynthetic pathway beyond PBG.

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Clotten is correct, the principal question which remains is why the patient had photosensitivity at all. With such a small amount derived from red cell destruction and no porphyrin demonstrable in the plasma, it is difficult to understand how sufficient porphyrin might accumulate in the skin to produce photodermatitis. Obviously of paramount importance to this question is the microfluorospectrophotometric study of skin biopsies such as Runge has carried out in protoporphyria (33).

From the foregoing it will be evident that further study of additional cases of this type is much needed.

Let me close with all good wishes to my friend Jan Waldenström on the occasion of his sixtieth birthday and the hope that he will have many years of health, and happiness in his work and with his family.

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domonis Spheroides in mixture II of Lascelles containing ALA as substrate (Gajdos and Gajdos Török 1963 a)

Our experiments have shown that ATP reduces the porphyrin biosynthesis probably by the enhancement of the formation of a physiological inhibitor. Our observations seem also to show that this inhibitor favours the utilisation of succinate for metabolic pathways others than that of ALA synthesis (Gajdos and Gajdos Török 1963 a)

These findings have led us to study in rats the effect of administration of compounds capable of reducing the biosynthesis of ATP. We have thought that if our earlier conclusions are correct these compounds would deter mine an excess formation of porphyrins.

Three chemically highly different compounds were utilised: 6-mercaptopurine, orotic acid and ethionine. They were administered in separate experiments to female adult rats maintained in metabolic cages on balanced diet when not otherwise stated.

We summarize briefly our observations published elsewhere in details.

I. 6-mercaptopurine was given by gavage in a daily dose of 20 or 40 mg/kg during 4 weeks (Gajdos and Gajdos Török 1963 b).

The biosynthesis of adenine nucleotides was controlled in the red cells. As it is shown in fig. 1, a statistically significant decrease was observed in the level of ADP and ATP with an equally significant increase in the concentration of AMP.

It seems therefore that intoxication with 6-mercaptopurine has led in our experimental conditions to the lowering of the biosynthesis of ATP by partly reduction of the phosphorylation of AMP.

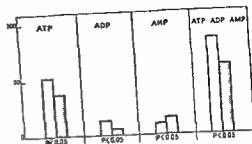


Fig. 1 Levels of purine nucleotides in red blood cells ($\mu\text{g}/100 \text{ ml}$) of normal rats and of rats intoxicated with 6-mercaptopurine (2 mg/rat/day) during 2 weeks.

White bars mean values of 9 normal rats. Shaded bars mean values of 4 rats intoxicated with 6-mercaptopurine.

As to the porphyrin synthesis we have noted the stimulation of urinary excretion of ALA and coproporphyrin (in one experiment for instance the mean values were raised from 7.0 to 48.2 and from 1.2 to 14.0 $\mu\text{g}/\text{rat}/\text{day}$ respectively) without appearance of uroporphyrin in the urine. The increase in the hepatic level of ALA, Pbg and ether-soluble porphyrins (copro and protoporphyrin) was statistically significant.

The excess formation of porphyrins caused by intoxication with 6-mercaptopurine was significantly reduced by administration of AMP or inosine at the daily dose of 20 mg/rat.

II. For the study of effect of orotic acid on porphyrin metabolism this compound was mixed to a balanced

Excess porphyrin formation following administration of inhibitors of the biosynthesis of ATP

By A. GAJDOS (Paris)

The extremely low quantity of free porphyrins in comparison with the quantity of these pigments incorporated into hemoproteins (hemoglobin, myoglobin etc) indicates a highly effective regulatory mechanism of the biosynthesis of porphyrins. This mechanism is at present poorly understood.

We have recently observed, that ATP may play an important role in this point of view (Gajdos A. and Gajdos-Torok M. 1963 a).

In our studies on the effect of purine nucleosides and nucleotides on the synthesis of porphyrin by *Rhodospseudomonas spheroides* incubated semianaerobically in the light in medium I of Lascelles (containing glycine and succinate as substrates) we have observed an almost complete inhibition of the synthesis by addition of ATP to the incubation mixture at the concentration of 3 mM (Table 1). Incubation semianaerobically in light during 48 hours of *Rhodospseudomonas spheroides* suspended in mixture I of Lascelles (5.5 mg microorganisms

Table 1

	Dry weight of micro organisms mg/ml	Porphyrins formed $\mu\text{mM/ml}$	Bacterio- chlorophyll $\mu\text{mM/ml}$
Without addition	1.2	36.9	1.69
With adenine	1.1	29.5	1.25
With adenosine	0.9	14.6	0.84
With inosine	1.2	19.3	1.65
With AMP	1.2	7.9	1.35
With ADP	1.0	10.9	1.40
With ATP	1.1	0.5	1.35

in 10 ml). The purine nucleosides and nucleotides were added to the incubation mixture at the concentration of 3 mM.

The inhibitory effect of AMP and ADP was not only lesser but also slower than that of ATP indicating that the former nucleotides may act as precursors of ATP.

We have also established that the inhibition by ATP is located to the synthesis of δ -aminovulnic acid (ALA). In fact, the porphyrin synthesis was not modified when ATP was added to a suspension of *Rhodospseu*

Table 3

	Rats intoxicated with ethionine during 1 week	Controls
Liver ATP ($\mu\text{mol}/100$ wet w)	79 ± 7.0 (mean of 14 rats)	168 ± 11 (mean of 14 rats)
	$t=6.3 \quad P<0.01$	
Ethersoluble porphyrins in liver ($\mu\text{g}/100$ wet w)	171 ± 2.3 (mean of 12 rats)	11 ± 1.0 (mean of 12 rats)
	$t=2.3 \quad P<0.05$	
Ethersoluble porphyrins in red blood cells ($\mu\text{g}/100$ ml)	61 ± 7.2 (mean of 12 rats)	33 ± 3.5 (mean of 12 rats)
	$t=3.6 \quad P<0.01$	

coproporphyrin from 3 to 177 proto porphyrin from 21.4 to 492.3 $\mu\text{g}/\text{g}$ dried feces

We have noted a highly significant decrease in the hepatic level of ATP and a similarly significant increase in the concentration of ethersoluble porphyrins in the liver and red blood cells (table 3)

We have not found an increase in the activity of ALA synthetase in the liver mitochondria Granick (1963) has recently observed that compounds capable of inducing enhanced porphyrin synthesis determine the de novo formation of the enzyme in the mitochondria of hepatic cells. Our negative findings after administration of ethionine may be explained by the inhibition of protein synthesis by this toxic agent as it was shown by Farber et al (1964) and Weber et al (1964)

Administration of AMP or inosine or ATP did not reduce in our experiments the excess of porphyrin formation caused by intoxication with ethionine. This inefficacy of adenosine nucleosides and nucleotides — the only one we have observed in the numerous

experimental porphyrias — may be explained by the inhibitory effect of ethionine on protein synthesis. In fact our experimental findings have indicated that the effect of ATP on excess porphyrin biosynthesis consists in the stimulation of the formation of a physiological inhibitor which our observations have shown to be proteinic in nature

In every case the determinism of an experimental porphyria in rats by administration of three different compounds — 6 mercaptopurine, orotic acid and ethionine — which have the common character of being inhibitors of ATP biosynthesis furnishes further arguments in favour of the regulatory role of this nucleotide in the biosynthesis of porphyrins

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Table 2

	Rats intoxicated with orotic acid during 3 weeks	Controls
Liver ATP ($\mu\text{mol}/100 \text{ g wet w}$)	60 ± 8.7 (mean of 10 rats)	161 ± 11.0 (mean of 10 rats)
	$t=16 \quad P < 0.001$	
Hepatic level of etherosoluble porphyrins ($\mu\text{g}/100 \text{ g wet w}$)	16.2 ± 0.9 (mean of 8 rats)	3.1 ± 1.4 (mean of 6 rats)
	$t=4.0 \quad P < 0.01$	
Etherosol porphyrins in red blood cells ($\mu\text{g}/100 \text{ ml}$)	690 ± 3.5 (mean of 8 rats)	306 ± 1.90 (mean of 10 rats)
	$t=2.6 \quad P < 0.001$	
Etherosol porphyrins in pooled bone marrow ($\mu\text{g}/10^{10}$ red blood cells)	38 (mean of 8 rats)	23 (mean of 8 rats)
ALA synthetase activity in liver mitochondria ($\mu\text{mol ALA formed}/\text{mg protein of mitochondria}$)	19.8 ± 3.0 (mean of 4 rats)	0 to 4.5 (10 rats)

dict at the rate of 1 % (Gajdos and Gydos Török 1965b)

During the 3 weeks of administration of orotic acid, the mean value of fecal etherosoluble porphyrins raised from 50 to 313 $\mu\text{g/g}$ dry feces. On the contrary, the urinary excretion of porphyrins and porphyrin precursors was not significantly modified.

At the end of the third week, the animals were killed. In the liver, a highly significant decrease in the level of ATP was observed confirming the findings of Von Euler et al (1963). The concentration of etherosoluble porphyrins was raised in the liver, red blood cells and bone marrow. On the other hand, an important increase in the activity of ALA synthetase was noted in the liver mitochondria.

As in the intoxication with 6-mercaptopyrimine, the excess porphyrin formation caused by orotic acid was significantly reduced by administration

of AMP or inosine at the daily doses of 20 mg per rat.

III *Ethionine* was shown by Stekol et al (1960) and others to strongly lower the hepatic level of ATP. Gibson et al (1962) reported that this amino acid stimulates porphyrin synthesis in *Rhodopseudomonas Spheroides*. Thus, the utilisation of this compound in our experiments was particularly interesting.

We report here only the result of one of our experiments (Palm Carlos et al 1965) where a daily dose of 50 mg ethionine per rat was administered by gastric tube during one week to rats maintained on relatively low protein diet (12 % casein).

The renal excretion of ALA and coproporphyrin was raised at the end of the intoxication respectively from 7.7 to 19.5 and from 0.5 to 3.6 $\mu\text{g}/\text{rat/day}$. An even more clearcut increase was noted in the level of fecal porphyrins.

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	Rats intoxicated with ethionine during 1 week	Controls
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The Relationship between the Neurological and Biochemical Lesions in Acute Intermittent Porphyrria

By C H GRAY

Until nearly a decade after the publication of Waldenström's monograph on porphyria (31) the nervous system lesions on the one hand and constipation with abdominal colic on the other were accepted as due to a toxic action of porphyrins on the nervous system and the gut respectively. This view received some support from early pharmacological studies using impure preparations of porphyrins although Waldenström himself mentioned the possible importance of vasospasm in causing the abdominal abnormalities. Lowry, Schmid, Hawkinson, Schwartz & Watson (19) suspected that porphobilinogen or a closely related substance might be responsible for the clinical manifestations. However, Goldberg, Piton & Thompson (14) showed that purified porphyrins and porphobilinogen are pharmacologically inactive and produced evidence against the possible existence of a circulating vasoconstrictor substance in acute porphyria. Later, Jarrett, Rimington & Willoughby (18) showed that 6-aminolavulinic acid, the biosynthetic pre-

cursor of porphobilinogen excreted in large amounts in the urine in acute porphyria, was similarly without pharmacological action. Goldberg (13) suggested all the clinical features of acute porphyria could be explained on a neurological basis, the gastro-intestinal features being attributed to lesions of the preganglionic motor fibres that innervate the viscera. Degenerative changes were found in the nuclei of nerve cells in the spinal cord and medulla which give rise to these fibres.

The earlier work on the changes in the nervous system in acute porphyria have been admirably summarised by Heirons (17) who confirmed earlier observations of Denny Brown & Scarra (7) and others in demonstrating abnormalities in the anterior horn cells but disagreed with their concept that the nerve lesions were secondary to vascular abnormalities. Heirons considered that the vascular lesions occurring in the brain were probably secondary to the severe hypertension which is characteristic of the disease and that the mental and neurological

disturbances were due to a direct, possibly reversible, metabolic effect of the disease resulting in some affection and loss of neurones in the brain and cerebellum and spinal cord Goldberg (13), however, had emphasised the importance of primary demyelination.

The controversy concerning the histopathological changes has been the subject of a recent annotation in the *Lancet* (2). The peripheral neuropathies may be due to a primary abnormality of the axon or to primary damage to the myelin. In the former there is a "dying back" process in the peripheral nerves and the demyelination is secondary to it, in the latter the demyelination, which is segmental and patchy, is in the Schwann cells and nerve sheaths and results in the axon degeneration. Erbsloh (9), Mason, Courville & Ziskind (20) and Simpson (25) all found evidence suggesting that the damage to the peripheral nerves in acute porphyria affected primarily the axons. On the other hand, Denny-Brown & Scriver (7) had observed segmental demyelination in the peripheral nerves of two patients while Gibson & Goldberg (12), in a study of peripheral and autonomic nerves, found demyelination occurring earlier to a greater degree than in the damage to the axons. More recently Cavanagh & Mellick (4) have examined the muscles, peripheral nerves, spinal cord and root ganglia in four subjects with porphyria. They correlated the clinical features of their cases with the extent of the lesions and with the distribution of the motor and sensory nerves in the affected muscles.

Regardless of the period of paralysis, there is gross denervation of muscle, the degeneration in the nerves being of the dying back form characteristic of primary axonal disease. Unlike the neuropathy of organophosphate poisoning in which the distal muscles with the longest nerve fibres are affected earliest and most extensively, the dying-back in acute porphyria occurs irrespective of the distance of the muscle from the spinal cord. The severity of the weakness will therefore depend upon the size of motor unit affected. In addition to the motor neurones which are particularly affected, sensory fibres in the nerves and sensory roots were involved although there was no clinical indication of sensory disturbances. Heirons (17), and Goldberg (13) had previously shown that sensory nerves may be affected. Cavanagh & Mellick (4) emphasised our ignorance of the rate of ascent of the lesions and point out that the histopathological changes may only be obvious in those subjects surviving longest. They suggest that in those subjects in whom recovery occurred the distances over which regeneration of the nerve takes place must be relatively short.

There is now little doubt that all the clinical features of acute intermittent porphyria are directly due to changes in the nervous system. Goldberg (13) postulated the formation of a substance λ of which porphobilinogen was a precursor essential for nutrition of myelin of the nervous system. He postulated that in acute porphyria there was a metabolic block in the for

mation of Δ which leads to an increased excretion of porphobilinogen and also causes demyelination. A primary defect in the motor neurones with a lesser abnormality in the sensory neurones could not be due to such a disturbance of myelin metabolism but might follow from an abnormality of acetylation and succinylation. De Matteis and Rimington (6) proposed a deficiency of acetyl choline synthesis due to an increased competition for glycine due to succinyl coenzyme A. The condensing enzyme responsible for the condensation of succinyl coenzyme A with glycine to form Δ can also bring about the condensation of acetyl coenzyme A with glycine to form amino acetone. If for some reason the rate of production of acetyl coenzyme A were limited the resulting deficiency of acetylcholine could interfere with transmission of the nerve impulse and larger quantities of succinyl coenzyme A might react with glycine to form Δ . Such a theory would suggest that amino acetone is a normal metabolite in the human subject and that amino acetone would be absent from the urine of patients with acute porphyria. However Tschudy, Welland, Collins & Hunter (28) Drury & Hunter (8) found no differences between the amino acetone excretion of patients with acute porphyria and that of normal subjects.

Rimington (24) has pointed out that many of the features of acute porphyria could be attributed to defective synthesis of acetylcholine because of interference with the acetylating

mechanism, a deficiency of coenzyme A or of acetyl coenzyme A from pyruvate or of available energy from ATP. The effect of barbiturates on porphyria could be due to the effect of this drug in uncoupling oxidative phosphorylation which would decrease the availability of ATP for the synthesis of acetyl coenzyme A. This might provide a basis for the apparent benefit of adenosine monophosphate or inosine phosphite treatment of acute porphyria [Gajdos & Gajdos Török (11)]. However Urata & Granick (29) have shown that two distinct enzymes are responsible for the synthesis of aminooctone and Δ respectively.

There has been much speculation as to whether the excessive excretion of Δ and PBG was due to an overproduction or to under utilisation of Δ . There might be either a block in the catabolism of one of the precursors of Δ via an alternative pathway not leading to Δ or PBG or to a block in the metabolism of Δ itself. The site of such a block has not been identified. Richards & Scott (22) showed that it was unlikely that any of the major pathways of glycine metabolism were blocked and they attempted to assess the magnitude of the Shennith pathway by which Δ is decarboxylated to give α -oxoglutaraldehyde which would then be degraded to succinic acid and a one carbon atom fragment. They investigated the ability of the body to convert glycine to serine a process requiring such a one carbon atom fragment but showed that in only 3 of 6 patients with

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acute porphyria may not be caused directly by the formation of ALA and PBG a metabolic event perhaps in the nervous system or elsewhere might be responsible both for the nervous system lesion and for the excretion of the metabolites in excess

Until the precise metabolic abnormality is known and the precipitating features identified the absence of correlation between ALA and PBG excretion and clinical symptoms and the great variation in the clinical picture in the disease cannot be understood. In some patients the motor nervous system is affected and they are paralysed in others the autonomic nervous system is mainly affected and there is abdominal pain. The central nervous system abnormalities develop dramatically reaching their maximum within a few hours. The patient may then be left with residual abnormality even though there has been recovery from the metabolic disturbance responsible. Such acute changes followed by return to the metabolic state characteristic of remission may account for the inconsistent results obtained by Richards & Scott (22) in investigating the conversion of glycine to serine and also account for the observations by Smith & Faylor (26) that the α -oxoglutarate concentration in the blood of patients with acute porphyria was increased in only 4 out of 5 subjects and the failure of others to confirm their findings. There is urgent need to re-investigate many of these problems during the acute stage of the disease and not at a time when a spontaneous

recovery and remission is probably taking place.

The similarity between the neurological disturbances in lead poisoning and those characteristic of acute porphyria and the increased excretion of ALA and coproporphyrin III in the former condition suggests that a more detailed study of liver and brain metabolism in both would be worthwhile particularly because experimental porphyria in animals is never accompanied by lesions of the nervous system whereas these are characteristic in experimental lead poisoning.

Carbohydrate has been shown to inhibit the induction of ALA synthetase of ALA in experimental animals [Tschudy Welland Collins & Hunter (28)] and also to reduce the excretion of ALA and PBG by patients with acute intermittent porphyria [Welland Hellman Gaddis Collins Hunter & Tschudy (33)]. These workers discuss their findings in relation to the catabolite repression of enzyme induction in which catabolites which are formed rapidly from glucose accumulate in the cell and repress the formation of enzymes whose activity would augment the already large intracellular pools of these compounds. They found it difficult to invoke the concept of catabolic repression to explain the effect of glucose on ALA synthetase. The important role of glucose in brain metabolism would make it highly desirable that this repressive effect of carbohydrate should receive careful re-investigation. Perhaps the elegant investigations now being carried out by Gaudonde (10) Balazs (3) and Vrbas

acute porphyria was the conversion of glycine to serine abnormally low. These findings require confirmation and further investigation.

The primary metabolic lesion in acute porphyria has still not been identified despite the demonstration that in the experimental porphyrias induced by allylisopropylacetamide (AIA) or diethylhexylhydrocollidine (DCC) there is an induction of a greatly increased synthesis of the enzyme ALA synthetase [Granick & Ulatz (15)]. They have postulated that these porphyrinogenic agents act in antagonising a repressor gene controlling the formation of ALA. More recently Perlroth, Tschudy, Marver, Collins, Hunter & Rechcigl (21) have shown that in acute intermittent porphyria in human subjects the hepatic ALA synthetase is greatly increased. They agree that this provides some support for the view of Watson, Runge, Tiddeim, Bossenmaier & Cardinal (32) that there is a constitutive operator mutation for ALA synthetase and that the neurological manifestations are a direct consequence of the over production of ALA and PBG. They point out however that the condition might be due to a defective structural gene lying outside the porphyrin biosynthetic pathway and that the induction of ALA synthetase is a secondary phenomenon. The primary defective structural gene would then lead to an enzymic deficiency and predispose the patient to the clinical features of the disease.

Many workers have failed to appreciate the great capacity for recovery

shown by patients with severe nervous system lesions in acute porphyria. The histological findings described by numerous authors may account not for the acute lesions but for the residual abnormalities of the central nervous system which characterises many patients between relapses. This reversibility of the major neurological disturbances associated with an acute attack provides important support for Heiron's concept that the vascular lesions reported by Denny-Brown & Sciarra (7) were probably secondary to hypertension and that the mental and nervous system disturbances were due to direct and probably metabolic effects of the disease comparable to the effects of mescaline and similar drugs. Ackner, Cooper, Gray, Kelly & Nicholson (1) emphasised the absence of correlation between PBG and ALA excretion and the clinical symptoms and reiterated the view that the excretion of the two metabolites and the nervous system changes are separate manifestations of a metabolic event or series of events. However they admitted that it was possible that a severe acute attack with neurological manifestations might be associated for a short period with a sudden burst of excessive excretion of the two porphyrin precursors. The association of neurological symptoms and signs in acute porphyria with the excretion of ALA and PBG in the urine may be analogous to the relationship between diabetic coma and ketosis. Coma may be accompanied but not necessarily caused by the production of ketonic acids and similarly the neurological lesions of

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(30) at the Medical Research Council Neuropsychiatric Research Unit in London, could be extended to investigate the effect of any of the porphobilinogenuric drugs on brain metabolism. They have shown that although in abnormal animals a part of $[U-^{14}C]$ glucose is metabolised directly via the tricarboxylic cycle to carbon dioxide, a substantial part is retained in the brain as amino acids, proteins and lipids. There was a particularly high incorporation into glutamate which not only undergoes transamination to α -oxoglutarate but is oxidised via glutamate aminobutyric acid (GABA) and succinic aldehyde. They calculate that in the steady state the turnover rate of glucose through GABA was about 10% of turnover through the tricarboxylic acid pathway. An investigation of the metabolism of glutamate and GABA in acute porphyria might well be rewarding.

Liver lipid phosphorus and total lipid have shown to be increased in sedormid porphyria [Schwartz (24)] as is fatty acid synthesis in the liver of ALA treated rats. More recently Taddem, Nordstrom & Wilson (27) have found a marked elevation of cholesterol, total lipids and phospholipids after ALA and DCC in rabbits. Gray & Waterfield (16) have also shown that two porphyrinogenic agents profoundly disturbed the incorporation of ^{14}C from $[U-^{14}C]$ glucose into protein and glycogen. However De Matteis (5) has shown that some of the metabolic changes which accompany the disturbance of ALA and PBG synthesis can also occur after the administra-

tion of related drugs not producing experimental porphobilinogenuria. He therefore, believes that the association of these changes with increased porphyrin formation should be regarded as coincidental. There is clearly urgent need to investigate further the metabolic pathways either directly or indirectly related to ALA synthesis in both brain and liver and perhaps other tissues of the body.

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Erythropoietic Protoporphyrria

A study of known cases in Sweden

By BIRGITTA HAEGER ARONSEN and GÖSTA KROOK

Protoporphyrria erythropoietica (P.E.) became the focus of extensive interest after its rediscovery in 1961 by Rimmington and coworkers, who gave the disease its name (17). It had been described as early as 1953 in Germany (12) but had for some reason apparently fallen into oblivion.

Clinically, the disease is characterized by an abnormal sensitivity to light, which usually makes its first appearance within the first two years of life. A few minutes' exposure to sunshine is enough to cause intense painful itching in the area exposed. This is followed by erythema, oedema and usually crust formation especially on the nose, cheeks and ears. Occasionally eczematous changes occur. The skin becomes coarse and affected children then look old for their age. This is thought to depend on the presence of large amounts of hyaline material deposited around the blood vessels of the upper corium (20). The nails are often opaque and have no lunulae. Vesicles, hyperpigmentation

and hirsutism sometimes develop though rarely.

The laboratory findings are dominated by a markedly increased concentration of protoporphyrin in the red blood cells, the plasma and the faeces. The faecal coproporphyrin content may be slightly increased while the urinary porphyrins and their precursors, δ -aminolaevulinic acid and porphobilinogen, occur in normal amounts.

Microscopic examination in ultraviolet light of erythrocyte smears shows abundant bright red fluorescing cells.

Some 50 cases of P.E. have been reported in countries outside Scandinavia. Most cases have been seen in U.S.A. (9, 10, 21, 23—26) but several also in Great Britain (3, 11, 17), Germany (12—15) and France (1—2). One case has been described in South Africa (27). The frequency of the disease does not vary with sex. In the first German and British cases no family studies were made, which we had

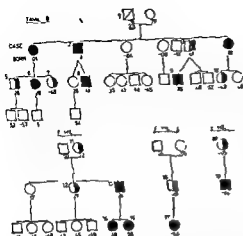


Fig. 1 Four families with protoporphyria erythropoietica. Squares represent males and circles females, solid symbols manifest cases, semi solid symbols latent cases, hollow symbols unaffected members. Diagonal across symbol indicates that the members has not been examined.

the opportunity to make in our first case (7-8). We showed that the disease is genetically determined and that its heredity is probably dominant. This assumption was later corroborated by others and by further personal investigations. It is known for instance that a pair of American identical twins were affected with the disease (2).

Material

Our Swedish material consists of 24 cases belonging to 5 families (Fig. 1). One (family B) of them can be traced back to Norrland, the northern part of Sweden from where also most cases

One family (cases 20-24) will be published later by Dr. H. Hansson and Prof. N. Thyresson, Uppsala, Sweden.

of acute intermittent porphyria have been reported (29-30). Genetical studies in which the ancestors were followed back to the beginning of the 19th century however failed to reveal any blood relationship between this family and families with acute porphyria.

Of our 24 cases half occurred in males and half in females. Fifteen cases were clinically manifest while the remaining 9 were asymptomatic but the patients had an increased concentration of protoporphyrin in the red blood cells (7) or in the faeces (2). The 9 asymptomatic cases were classified as latent.

Case reports

Family B

Case 1 S J. 64 year-old woman, housewife. Since childhood she has suffered from discomfort, almost nausea on exposure to sunshine. No other general symptoms. The skin becomes red but not tanned. Grey blue eyes previously fair hair now grey.

Case 2 C B. 62 year-old man, Unskidded labourer. Since 20 years of age he has had itching of the skin on exposure to sunshine. He reported that he also may develop pepper corn sized blisters which heal without scars. The cutaneous changes only appear on the hands, never in other areas of the skin, not even after long exposure to sunshine. The patient tans readily. Blue eyes, brown hair.

Case 3 L W. 47 year-old woman, Housewife. Has never liked to sunbathe. Cannot tolerate sunshine but is unable clearly to define the symptoms. On exposure to the sun the skin never tans but only turns temporarily red without other symptoms. On one occasion in the spring of 1963 when the patient was in the mountains the face became red and swollen. No vesicles or peeling of the skin. She recovered after one week. Grey brown eyes, hair previously fair now grey.

Case 6 B O 37 year old woman Clerk Her skin becomes red on exposure to sunshine Never tans Never itching oedema or other skin symptoms No discomfort on exposure to the sun Grey blue eyes, brown hair

Case 6 R B 24 year old man Shop assistant Abnormally sensitive to sunshine already at one month of age After about five minutes exposure in summer or about 30 minutes in winter the skin begins to itch with a sensation of burning and pricking in the exposed areas Sometimes he also has chills which continue for days and disturb his sleep The itching is followed some hours later by oedema and erythema After severe exposure pinheadsized vesicles occasionally occur When after 1—3 days the oedema has abated fissures form which heal leaving behind small scars The skin on the back of the hands is sometimes yellow white or blue yellow Sometimes the skin lesions are accompanied by watering of the eyes some hours after exposure to sunshine he may pass 2—3 loose dark stools and sometimes also big volumes of urine which however looks normal The symptoms are not confined to any particular season of the year but are most marked between February and August Grey blue eyes dark hair

Case 9 B B 30 year old man Engineer Abnormally sensitive to sunshine since 6 years of age Exposed areas of the skin feel irritated burn and are tender No itching Sometimes erythema oedema and petechiae occur On one occasion only when he was in the mountains the skin blistered No crusts or scars develop No hypertrichosis or increased brillianess of the skin Normal pigmentation after exposure to sunshine Exposure has never caused any systemic symptoms such as chills or nausea Grey blue eyes brown hair

Family R

Cases 13 14 and 15 correspond to cases 2 12 and 13 in a previous publication (8)

Family L

Case 17 C L 10 year old girl Since 4 years of age the patient has had itching pain and sensation of burning in the skin in areas ex-

posed to sunshine These symptoms are often accompanied by chills She occasionally has oedema but never erythema Small pale milia sometimes appears but no blisters Crusts form and peel after a few days When after 1—2 months they have fallen off they leave behind small irregular scars Grey blue eyes fair hair

Family V

Case 19 L A 21 year old man Electrician Since 3 years of age he has had itching and sensation of burning in sun exposed areas of the skin Erythema and diffuse oedema as well as chills sometimes occur and only on a few occasions has the skin blistered but never was there left behind any scars Does not tan readily not even in summer Grey blue eyes very fair hair

At the examination all 8 cases reported appeared physically and mentally normally developed The dental status was also normal Neither the liver nor the spleen was palpable In none of them the skin was brittle Case 2 is hyperpigmented all the year round The patient in case 8 was moderately pigmented over the metacarpophalangeal joints especially II—III bilaterally He had several small scars in the forehead on the tip of the nose and on the cheek The nails of the second and third fingers on either hand had no lunulae In case 9 the patient had numerous lentigines on the arms and back but otherwise showed nothing remarkable Case 17 showed small irregular depressions on the condyles of the second and third fingers on either hand and dirty brown pigmentation of the backs of the hands and lichenification of several of the knuckles Scaling of skin on the tip of the nose giving follicular hyperkeratosis

scabs and a few scars. Case 19 also showed lichenification of several knuckles but no other skin lesions. All the nails were thick and had no lunulae.

Three of our patients (nos 8, 9, 19) reported that the more often they are exposed to sunshine the quicker the symptoms appear (a loading up?). In skin tests (with Kromayer's lamp equipped with monochromatic filter Zeiss 508953) on one of our patients (no 19) itching appeared as well as a sensation of burning in areas previously exposed to sunshine already after 10 minutes but not until after three times this interval in areas not previously exposed.

Four patients (nos 2, 8, 9, 19) reported that dirt and sweat on the skin can accelerate and accentuate the symptoms. One (no 2) of them reported that he has symptoms only on the hands (dirt?) even when he also exposes other parts of the body.

It is remarkable that 3 of the patients (nos 1, 4, 6) had a hitherto undescribed relatively mild form of P I where the only symptoms were red dening of the skin, sometimes intense without subsequent tanning, nausea and general feeling of discomfort on exposure to sunshine.

Four patients (nos 8, 13, 17, 19) were often troubled by chills after exposure to sunshine, a symptom we have not found mentioned in other case reports of P I.

Another symptom not reported is the frequent passage of loose dark stools, which was noted in one of our cases (no 8).

Table 1 Concentration of protoporphyrin in red blood cells (PP/RBC), coproporphyrin in faeces (CP/F) and protoporphyrin in faeces (PP/F) in 11 manifest (M) and 8 latent (L) cases of protoporphyrin erythropoietic I.

Case		Time of analysis	PP/RBC $\mu\text{g}/100 \text{ ml}$	CP/F $\mu\text{g}/\text{g}$ dry weight	PP/F $\mu\text{g}/\text{g}$ dry weight
1	SJ	M	63.02	51	15
2	GB	M	63.02	57	7
3	VB	L	63.02	58	9
4	LW	M	63.02	53	13
5	HJ	L	63.02	11	12
6	BO	M	63.02	56	21
7	GL	L	63.02	23	20
8	RB	M	63.02	1944	18
9	BB	M	63.04	503	9
			03	830	8
10	KW	L	63.02	43	9
11	ER	L	62.01	50	0
12	MO	L	62.01	40	0
13	LR	M	62.01	839	5
14	BR	M	62.01	211	0
15	EH	M	62.01	763	2
16	IL	L	63.03	59	4
17	CI	M	63.03	384	—
			09	625	4
18	SV	L	63.03	61	6
19	LV	M	64.04	332	15
			63.04	403	7
			03	633	6
Normal upper limit in +2 SD			≤ 25	≤ 6	≤ 3

Results

The results of the most relevant analyses, protoporphyrin in red blood cells (PP/RBC), coproporphyrin in faeces (CP/F) and protoporphyrin in faeces (PP/F) in our series are given in table 1. All the analyses were performed by the methods described previously (1, 5, 6).

Case 11 B O 37 year old woman Clerk Her skin becomes red on exposure to sunshine Never itans Never itching oedema or other skin symptoms No discomfort on exposure to the sun Grey blue eyes brown hair

Case 6 R B 24 year old man Shop assistant Abnormally sensitive to sunshine all ready at one month of age After about five minutes exposure in summer or about 30 minutes in winter the skin begins to itch with a sensation of burning and pricking in the exposed areas Sometimes he also has chills which continue for days and disturb his sleep The itching is followed some hours later by oedema and erythema After severe exposure pinheadsized vesicles occasionally occur When after 1—3 days the oedema has abated fissures form which heal leaving behind small scars The skin on the back of the hands is sometimes yellow white or blue yellow Sometimes the skin lesions are accompanied by watering of the eyes Some hours after exposure to sunshine he may pass 2—3 loose dark stools and sometimes also big volumes of urine which however looks normal The symptoms are not confined to any particular season of the year but are most marked between February and August Grey blue eyes dark hair

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Family V

Case 19 L V 21 year old man Electrician Since 3 years of age he has had itching and sensation of burning in sun exposed areas of the skin Erythema and diffuse oedema as well as chills sometimes occur and only on a few occasions has the skin blistered but never was there left behind any scars Does not tan readily not even in summer Grey blue eyes very fair hair

At the examination all 8 cases reported appeared physically and mentally normally developed The dental status was also normal Neither the liver nor the spleen was palpable In none of them the skin was brittle Case 2 is hyperpigmented all the year round The patient in case 8 was moderately pigmented over the metacarpophalangeal joints especially II—III bilaterally He had several small scars in the forehead on the tip of the nose and on the cheek The nails of the second and third fingers on either hand had no lunulae In case 9 the patient had numerous lentigenes on the arms and back but otherwise showed nothing remarkable Case 17 showed small irregular depressions on the condyles of the second and third fingers on either hand and dirty brown pigmentation of the backs of the hands and lichenification of several of the knuckles Scaling of skin on the tip of the nose giving follicular hyperkeratosis

An observation arguing against a decreased consumption of PP in the synthesis of haem in these patients is the largely normal Hb, serum iron and TIBC values. The sideroblasts were not increased in marrow smears. Finally the disappearance and appearance rate of iron in patients in this study have been normal.

The other possibility, increased synthesis, has been tested by Porter (22) in bone marrow incubation tests. He showed that the immature red blood cells in these patients have an overproduction of protoporphyrin.

Contrary to what one might expect the increased concentration of PP in plasma is not due to leakage from the PP loaded red blood cells but at least partly to an overproduction of this porphyrin in the liver (11, 24).

Can the clinical symptoms be explained by the markedly increased concentration of PP in the red blood cells and plasma? Porphyrins have long been known to be photosensitizers (28) and in other types of porphyria where these compounds meet sunlight hypersensitivity to light occurs. On examination of cases with P.E. Magnus (16, 18) found the sensitivity of the skin to be greatest on irradiation with wavelengths around 400 m μ corresponding to the absorption maximum of the porphyrins. Magnus believed that the hypersensitivity of the skin in P.E. is due to the effect of the light on the PP laden red blood cells in the capillaries (18). If this were correct one should however expect a corresponding skin reaction in lead

poisoning where the concentration of PP in the red blood cells sometimes reaches the same level as in P.E. But no such reaction is seen. A more plausible explanation of the hypersensitivity to light would therefore be the increased concentration of PP in the plasma which occurs in P.E. patients but not in leadworkers.

Therapy

Various ointments have been tried in the prophylaxis of P.E. but none have so far proved satisfactory. We ourselves have used red veterinary petrolatum cream with 10 % *p*-amino benzoic acid and Contralum® (Hermal Chemie Humberg) all without success.

Since antimalarial drugs have proved to have light protective effect in case 17 we tried chloroquine (Nivaquine® May & Baker Ltd) 50 mg daily by mouth during the summer of 1961. The preparation was without any effect. In the summer of 1963 we changed to Triquin® (Winthrop) with a good effect. As this preparation contains not only chloroquine but also oxychloroquine and quinacrine we thought the latter component to be responsible for the favorable effect since it absorbs radiation particularly at the maximum of porphyrins i.e. about 400 m μ which chloroquine and oxychloroquine do not (19). In the spring of 1964 when the patient again had severe lesions she was therefore treated with Quinacrine® (May & Baker Ltd) alone in a dose of 50 mg every other day for 1 month. Treatment

No determinations were made of the plasma protoporphyrin. In all the cases the excretion of δ -aminolaevulinic acid, porphobilinogen, uroporphyrin and coproporphyrin in the urine was normal.

As to the other laboratory studies, the haemoglobin concentration and the red and white blood cell count were determined in 7 of the patients and 4 (nos 8, 9, 13, 19) were found to have a moderate hypo- or normochromic anaemia with a minimum Hb of 12.3 g/100 ml (no 8). The number of red and white blood cells was normal in all of the patients studied. The ESR was likewise normal in all 5 patients examined in this respect. The serum iron was normal in 11 patients examined while the TIBC was slightly increased in two (nos 8, 10). The serum haptoglobin and bilirubin were determined in 12 patients and were found to be normal in all. The serum electrolytes and the GOT were normal in all of the 5 patients studied in this respect. The serum GPT was measured in 12 and 2 of them (nos 8, 11) showed slightly increased values of 44 and 33 units ($NV \leq 30$ U) while the others showed normal values. The bromsulphalein retention test was found to be normal in 2 patients studied. Serum protein electrophoresis was done in 8 cases. In 4 of them the pattern was normal while 2 showed a slightly increased concentration of γ globulin (nos 1, 7), 2 a slight increase of α_2 globulin (nos 2, 7) and finally 1 had shown a moderately decreased concentration of β_1 globulin (no 4). The changes thus showed no uniform tendency.

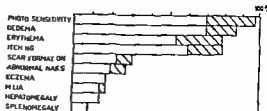


Fig 2 Frequency of clinical symptoms in 36 manifest cases of protoporphyria erythropoietica (1—3, 10—15, 17, 21, 23—27) including 11 personal cases.

Many of our patients were examined especially for haemolysis of the red blood cells (reticulocyte count, serum haptoglobin and bilirubin) in association with exposure to sunshine, but no haemolysis occurred. This is contrary to what was reported by Harris (10) and by Harber and coworkers (9) after *in vitro* studies of blood specimens from patients with PL, when they found a most striking haemolysis after 320—450 m μ radiation.

The frequency of different clinical symptoms are given in fig 2.

Discussion

Why then do the patients with PL have such a high concentration of protoporphyrin in the red cells, plasma and faeces? This porphyrin represents a normal intermediary stage in the haem biosynthesis. Once iron is incorporated in its core the synthesis is complete. An increased concentration of free PP in the red blood cells may occur either owing to a decreased consumption or increased production of this metabolite.

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had the desired effect and the patient could again tolerate sunshine. But 10 days after withdrawal she had a relapse, which was, however, soon controlled on resumption of treatment. Of 5 other patients (nos 8, 9, 13, 15, 19) treated with Quinacrine the drug produced the desired effect in only one (No 13).

Inosine, which Gajdos (1, 2) found to have a good effect on the symptoms of P.C., was tried in one of our cases (no 9) in a dose of 4 mg/kg body-weight (288 mg) a day, for 12 days, but without success. The treatment was, however, given for a much shorter period than that recommended by Gajdos.

Summing up, so far no really efficacious treatment of the symptoms of P.C. is available.

Summary

A survey is given of all known cases of protoporphyria erythropoietica in Sweden. Of the 24 cases hitherto diagnosed 15 are clinically manifest and 9 latent. The patients belong to five families and we have been able to confirm the previous assumption that the disease is inherited and that the gene is dominant. The clinical symptoms and biochemical abnormalities of the disease are described and the aetiology of the condition is discussed.

Acknowledgement

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examination if they had a hangover as this generally meant that they would be suspected of being lead poisoned.

Since 1961 the control of the workers at this factory has been performed by the author. Starting at the beginning of 1963 determination of the urinary output of delta aminolevulinic acid (ALA) was made in conjunction with the semi quantitative determination of coproporphyrins. Since 1964 coproporphyrins have not been determined and determination of ALA has been made routinely as suggested by Hegerl & Irons (4). Determinations of ALA give a good estimate of metabolically active lead in the organism (Grauer & Selander 5).

In the present investigation a comparison is made between the living habits of lead poisoned workers and those of workers with the same degree of lead exposure without symptoms of poisoning, to determine the influence of the factors mentioned above.

Materials and methods

ALA was determined as described by Grauer & Selander (5) in the modification of Hegerl & Irons (4). The results are compared with ALA excretion as μ 100 ml urine in the output of ALA excreted per gram of creatinine (Grauer & Selander 5). It was found that for routine purposes it is not necessary to take the output of creatinine in the work shift into account. Instead the values for freshly voided samples taken during the day are sufficient as the daily excretion of creatinine is about 100 ml urine. A value of 100 ml urine was calculated for all workers at the factory. The values were classified as being lead

Clinical material

Twenty six workers at the Tudor Street Battery Works shown in the previously described study of lead poisoning during 1964 were used. The workers were employed in the following work shifts of the plant (Table I). The ALA values for the two groups are given in table II.

Table I Workshops for lead poisoned and non poisoned workers

Ball mill and paste mixing	10
Formation	2
Filing	4
Lathe	6
Lead recovery and casting	4

For every lead poisoned worker a worker without signs of lead poisoning but with the same time of employment and the same degree of lead exposure was examined. In total 26 pairs of workers were examined. The oral examination as made by the author using a standardized questionnaire. The interviews were made with one worker at a time and in strict confidence. The subjects cooperated willingly and nobody refused to take part in the examination.

Results

The results are given in table II—A. There was no correlation between leisure time spent indoors and the use of tobacco in the workshop on the one hand and the frequency of lead poisoning on the other.

The difference in distribution of lead poisoning between those who ate one hot meal per day and those who ate two meals or more is at the border line of statistical significance. The difference between those consuming 75 cl or more of strong liquor per month and those with a lower alcohol consumption seems to be well established, however.

Predisposing Factors for Lead Poisoning

By KIM CRAWLER

The invention of semiquantitative methods to determine coproporphyrins in urine introduced in Sweden by Waldenström (1), marked an important improvement in the control of lead exposed workers. Since the beginning of the 1940's the principles worked out by Waldenström have been successfully applied in the control of lead workers.

The Tudor Storage Battery Works in Nöl, Sweden, the largest Swedish manufacturer of such batteries employ a total 500—600 workers of which an average of 200 are directly exposed to lead. During the period 1941—1960 the control of lead poisoned workers was made by the late professor Martin Odén. Experience gained during this period pointed to certain factors as potentially responsible for precipitating lead poisoning in the exposed workers. These were

oral cavity with a potentially high risk of simultaneous introduction of lead if the hands are not carefully washed. Lead contaminated pipe stems were also regarded as a definite risk.

2 *Dietary habits* It is known from previous Swedish dietary surveys, Odén (2) Söderberg (3) that those who eat one hot meal per day have a less satisfactory supply of essential nutrients than those who eat several hot meals. This could make them more liable to lead poisoning.

3 *Leisure time spent indoors instead of outdoors* Several workers were part time farmers owning a small land and spending most of their free time with harvesting and care of animals while others practiced fishing, cross country running or were active football players. The general assumption was that these workers were more seldom lead poisoned.

1 *The use of tobacco in the workshop* This applied especially to the use of snuff as snuff taking in Sweden means the introduction of about 5 grams of a tobacco mixture into the

4 *Consumption of alcohol* Notorious alcoholists were known to be liable to lead poisoning. In addition to this it was known among the workers at the factory that they should avoid in

sequently been replaced by a 75 cl bottle. Although ten years have elapsed since this partial prohibition ended, most Swedish men still can state their monthly purchase of liquor accurately, and there is no reason to distrust the correctness of the information given.

Liver damage of cirrhosis can result in an elevated urinary excretion of coproporphyrins while the excretion of ALA is normal (Haeger & Aronsen (4) Cramér & Selander unpublished).

The previously assumed high incidence of lead poisoning among regular consumers of liquor could be explained by an elevation of urinary coproporphyrins evoked by alcohol. This does not apply to the excretion of ALA which is not increased during or after acute alcoholism (Cramér & Selander unpublished results). The demonstrated difference in frequency of lead poisoning must therefore be related to some personal factor not to an interference in the porphyrin metabolism by alcohol. So far it can only be assumed that this factor is lack of personal hygiene or some other type of carelessness on the part of the regular alcohol consumers.

In conclusion the alcohol consumption of lead workers should be determined before employment. Those who admit that they are regular consumers of strong liquors should be placed in less dangerous position in the factory. Determinations of urinary ALA excretion should be substituted for determinations of coproporphyrins to avoid false diagnoses of lead poisoning.

Summary

Twenty six pairs of lead workers were examined. In each pair, one worker had shown signs of lead poisoning (ALA output in urine exceeding 1.5 mg/100 ml) and one had not. Time of employment in the factory and degree of lead exposure were comparable.

There was no correlation between smoking and/or snuff taking in the workshop and a higher incidence of lead poisoning. Nor did the leisure time spent indoors affect the frequency of the disease.

Those eating one hot meal per day had an almost statistically significant lower incidence of lead poisoning than those eating several meals.

A definite difference in the incidence of lead poisoning was found between those consuming alcohol regularly (75 cl of strong alcohol per month or more) and those with lower liquor consumption. $\chi^2 = 2.996$ $p < 0.02$.

These results are discussed and some practical conclusions are drawn.

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Table II *Mean values and ranges for ALA/100 ml urine in 26 lead poisoned and non-poisoned workers*

		Lead poisoned	Non poisoned
ALA mg/100 ml	mean value	4.20	0.77
	range	1.50-8.40	0.32-1.35

Table III *Use of tobacco in working hours*

	None	Cigarettes	Pipe	Snuff
Lead poisoned	10	8	3	5
Not poisoned	11	4	3	8

No significant differences

Table IV *Hot meals per day*

	1 meal	2 meals	3 meals
Lead poisoned	6	17	3
Not poisoned	15	9	2

$\chi^2 = 5.112$ $p < 0.05$ (1 meal vs 2 or 3 meals)

Table V *Leisure time spent outdoors*

	None	Only summer	The whole year
Lead poisoned	7	9	10
Not poisoned	4	8	14

No significant differences

Table VI *Amount of liquor purchased monthly*

	None	37 cl	75 cl or more
Lead poisoned	8	8	12
Not poisoned	17	6	3

$\chi^2 = 3.996$ $p < 0.02$ (none+37 cl vs 75 cl or more)

Discussion

There seems to be no comparable study performed before, and the results are therefore representative only for the factory in question.

One reason for the study was to provide a background for a formal ban on the use of tobacco. No correlation between the use of tobacco and the incidence of lead poisoning was found, however, and it must be postulated that the smokers and snuff takers generally observed the rules of a thorough hand washing before using cigarettes, pipes or snuff.

The difference between those who ate one hot meal and those who ate more than one hot meal per day is contrary to the expected one. It should once more be stressed that the difference lies on the very border line of significance, and that it therefore should not be over emphasized. One possible explanation would be that most of the one meal eaters took their meal in the factory canteen while the others generally ate sandwiches in the dry time with a potential danger of simultaneous introduction of lead, owing to a direct contact between the food and more or less lead contaminated hands.

The living habits during leisure time exerted no influence on the incidence to lead intoxication and the previous assumptions of the importance of an abundance of fresh air could not be verified statistically although the figures demonstrate a weak trend in this direction.

The principal finding however is the higher incidence of lead poisoning among those who consumed more than 75 cl alcohol per month. Liquor was rationed in Sweden until 1955 with a monthly ration of 1-3 litres per adult. The one litre bottle has sub-

II

PROTEIN DISTURBANCES

II

PROTEIN DISTURBANCES

Current Trends in Immune Globulin Research

By H. G. KUNKEL, J. HILLANDER and M. MANNIK

The essential importance of studies with myeloma proteins and Waldenström macroglobulins for elucidating problems of normal immunoglobulins and antibodies is belatedly receiving widespread acceptance. It is for this reason that we should particularly honor the individuals who first provided us with these useful tools — Bence Jones as well as his clinical cohorts Watson and McIntyre in 1845 and Professor Waldenström exactly 100 years later.

It has become an established sequence of investigative events to first make an observation concerning some characteristic of myeloma proteins or Waldenström macroglobulins, then to observe the same thing among the normal immunoglobulins and finally to distinguish it as a characteristic of one or another isolated antibody. No exceptions have been found concerning the similarity of these proteins and antibodies although it is important to continue to be alert to this possibility.

One example might be cited where an observation on a Waldenström macroglobulin initiated a most fruitful approach to the study of antibodies. This was the observation of Deutsch

(1) that these macroglobulins could be readily dissociated with sulfhydryl compounds. This was then followed by the finding from several laboratories that 19S antibodies are inactivated by such compounds and many investigators are now utilizing this sensitivity to distinguish such antibodies. However the value of Deutsch's initial observations extended considerably beyond its use among macroglobulins. It provided the stimulus that led Edelman in our laboratory to attempt dissociation of γG or 7S γ globulin with these compounds. This of course led to the definition of the L and H chains and thus markedly influenced the trend of experimental work on the chemistry of antibodies.

Many other examples could be given. The entire definition of the major classes of immunoglobulins rested primarily on the use of myeloma proteins and Waldenström macroglobulins. As Professor Waldenström has so long emphasized these proteins should not be termed paraproteins or pathological proteins. In fact they may well be individual antibodies closely resembling the presumed antibody produced by the cell from which the ma-

lignant clone developed. Such a concept is supported by the finding of a number of antibody like activities among Waldenström macroglobulins. Cold agglutinin activity and rheumatoid factor activity (2) represent two that are found in a number of instances. Particularly striking has been the case where a Waldenström macroglobulin agglutinated old cells (3). In the case of the myeloma proteins as well a few activities have been encountered. Among these is the anti streptolysin activity (4). Another is the property of certain myeloma proteins to combine with lipoproteins raising the possibility that they might be antibodies to lipoproteins. Certain of these patients show lipid deposits in their tissues which are surrounded by plasma cells suggesting the possibility of local proliferation of cells reacting in part to the stimulus of perhaps some abnormal lipid material. The problem is complicated by the tendency of certain myeloma proteins to link with a variety of serum proteins such as albumin through disulfide bonds. It is of particular interest that in most instances where antibody like activities have been encountered in Waldenström macroglobulins or myeloma proteins, they have had specificity for some autologous tissue constituents.

Classes of Immunoglobulins and Subgroups

Antigenic studies with myeloma proteins through the use of both rabbit and monkey antisera to such isolated proteins have proven very useful in

the elucidation of the various classes and subclasses of immunoglobulins. The general consensus is that there are now four major classes — γG , γA , γM and γD — which possess very different H chains and similar L chains (see review 5). In addition, there appear to be a number of subgroups due to H chain differences for each of the classes. This has been worked out fairly completely for γG globulin where at least four H chain subgroups have been characterized (We or γ_{16} , Ve or γ_{2a} , Vi or γ_{1c} and Ge or γ_1) (6, 7). Agreement concerning these subgroups has now been obtained in several laboratories.

A major problem in this field is the question of when to call a type of γ globulin a different immunoglobulin or simply a different subgroup since both classifications depend primarily on antigenic differences in the H chains. Up to now this division has rested primarily on the cross reactions obtained. A protein is a different immunoglobulin if there is no H chain cross reaction and a subgroup if cross reaction occurs particularly in the Fc fragment. However this question has become more confused through the recent observations that the different immunoglobulins do cross react partially in the H chains (8, 9). Furthermore the demonstration of separate genetic factors for the various H chain subgroups of γG globulin emphasizes their basic differences.

Figure 1 illustrates the procedure utilized in the definition of the H chain subgroups of γG globulin as applied to the example of the Vi or γ_{1c}

subgroup. Antiserum to a γ_1 type protein after absorption with most heterologous myeloma proteins of the major We group still reacts strongly with certain myeloma proteins (Δp , Fe and Hu) as well as with a fraction of normal serum and Fr II γ globulin. Myeloma protein We and most other myeloma proteins fail to react. Approximately 8 per cent of all myeloma proteins show this specific reaction. Quantitation of the component in normal γ globulin corresponding to these myeloma proteins has shown considerable variation in different sera in the range of 2—12 per cent of the total γ globulin. Immunoelectrophoresis experiments show a short distinct line under the major γG globulin line for all normal sera when unabsorbed antisera to γ_1 proteins are utilized.

The exact chemical basis for the H chain subgroups has not been clearly defined. Some carbohydrate differences have been encountered for certain of the subgroups particularly the Ge type where papain digestion reveals a uniquely fast migrating Fc component (6). Reduction with SH compounds indicates marked differences in the availability of the S-S bonds that are split. This is true for the γ_1 subgroup where papain digestion is also more rapid and carried further than with the other gamma globulins (10, 11). Differences in skin sensitizing capacity have been reported (12).

I Chain Variations

In addition to the well known subdivision of I chains and Bence Jones proteins into the H and L types (Group



Fig 1 The specific reaction of absorbed anti γ_1 antiserum with three proteins of the γ_1 subgroup (Δp , Fe, Hu) at 0.1 mg/cc as well as the reaction with a similar component in concentrated normal serum and in Fr II at 10 mg/cc concentration. A myeloma protein We of a different subgroup is shown for comparison.

I and II) further subdivision is readily possible by means of appropriate antisera (13, 14). Indications for such variation have long been apparent in our laboratory but have proven difficult to pin down into clearcut subgroups. Marked spurts can be demonstrated between different H type proteins with certain antisera. At least ten antigenic determinants have been distinguished among the H type proteins through the use of different discriminating antisera (14). All L chains or Bence Jones proteins examined could be distinguished with heterologous antisera as well as homologous antisera showing individual specificity. It was clear that more was involved than simple subgroups. This antigenic analysis was correlated with structural studies by Hilschmann and Craig on the same proteins (15) and it was apparent that these antigenic differences occurred primarily on the N terminal half of the proteins that is the portion

distal to the S S bond linking the L chain to the H chain. The antigenic differences were found within Inv(b+) type proteins and within Inv(a+) proteins and could not be correlated with the genetic types. A similar situation was found for the type L proteins although here the individual specificity was more marked. The results are perhaps best interpreted by assuming a number of different antigenic units which appear in various combinations in different Bence Jones proteins and L chains.

Individual Antigenic Specificity and the Variable Areas of the γ -globulin Molecule

Perhaps the most striking feature of the myeloma proteins from the antigenic standpoint is their capacity to elicit antibodies which are specific for the myeloma protein utilized for immunization. This property has long been known and has also been described for Waldenström type macroglobulins. It has received added significance in view of the finding of similar individual specificity for a number of isolated antibodies (16). Table I illustrates the individual specificity for three isolated anti A antibodies. The fourth anti A, W₁, failed to show such specificity and all antibodies were removed by heterologous absorption. In all respects these results were similar to those with isolated myeloma proteins.

One aspect of this problem that has aroused considerable interest is the question of whether this individual

Table I Reaction of 28 Isolated Anti A Antibodies with Rabbit Antisera to Four of the Isolated Antibodies

Isolated Anti A Ab	Antisera to Isolated Anti A Antibodies			
	Anti Th ¹	Anti Ka ¹	Anti Hb ¹	Anti Wa ¹
1 Th	+	0	0	0
2 Wa	0	0	0	0
3 Ka	0	0	0	0
4 Ar	0	0	0	0
5 27	0	0	0	0
6 Ka	0	+	0	0
7 63	0	0	0	0
8 64	0	0	0	0
9 66	0	0	0	0
10 Ho	0	0	0	0
11 Sa	0	0	0	0
12 Hb	0	0	+	0
13-28	0	0	0	0

¹ = Abs with Normal Serum (AB) + Fr II

specificity can be absorbed out by large amounts of pooled γ globulin. The concept behind such experiments is that the myeloma proteins have direct analogues in the spectrum of normal γ globulin molecules. Most investigations indicated that this was indeed the case. However, some results to the contrary have been obtained. Recent studies from our laboratories (17) and also by Seligmann and associates (18) indicate that in the vast majority of instances continued absorption with pooled γ globulin causes progressive removal of the specific reactivity. This has been brought out best by quantitative precipitation curves with the antisera absorbed with increasing amounts of γ globulin. In both laboratories however a few antisera have been encountered which

show little or no diminution of specificity by absorption with pooled γ globulin. The explanation for these exceptional findings remains obscure.

Considerable interest has focused on the areas of the molecule of the myeloma proteins and isolated antibodies at which the individual specificity resides. This area would obviously have important implications regarding the site of structural variation from one antibody to another. Initial studies demonstrated that this specificity always resided in the Fab fragment produced by papain (16) which also contained the antibody combining site. Further studies (17) with isolated H and L chains primarily from myeloma proteins demonstrated that with some antisera the H chains of the homologous myeloma proteins reacted specifically with others the L chains contained the specific antigenic sites. In the case of the H chains this specificity always resided in the Fd fragments and never in the Fe fragments. In some instances individual specificity could be localized to both the H and L chains. Of particular interest was the frequent finding that individual specificity required the combination of the two chains; neither chain alone carried the specificity. This was brought out best by recombination of the isolated chains.

Thus the localization of the individual antigenic specificity to the N-terminal half of the L chains and the Fd fragment of the H chains indicate that both these areas are subject to great variation from one myeloma protein to another and in all probabi-

lity from one antibody molecule to another. Chemical analyses from finger prints and amino acid sequence studies appear to substantiate these findings although much further work is required particularly for the Fd fragments where only limited studies are available (19).

The Genetic Factors of γ G globulins and their Molecular Localization

One very active area of investigation concerning the immune globulins involves the studies of the many genetic factors that have been discovered for these proteins. Antisera for the detection of these characters were initially obtained from rheumatoid arthritis patients (20) but at present the more useful source is from sera where isoimmunization has occurred in individuals receiving multiple transfusions or immunization by maternal γ globulin. Rabbit sera are also finding considerable use particularly in the search for new factors on proteins which at present lack any known genetic determinants (21). The myeloma proteins and Waldenström macroglobulins have played a particularly valuable role in the localization of the genetic factors to the chains of different immune globulins. The two characters (a, b) have been found on the L chains of all classes of immune globulins. However the known genetic factors of the H chains are thus far limited to γ G globulins and even here two of the H chain subgroups lack any known genetic factors. It is of special interest that the Gm(a) and Gm(b)

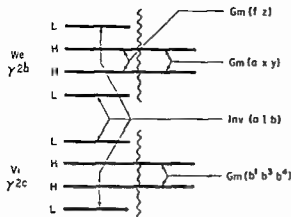


Fig. 2 Schematic diagram of the chains of γ globulin for the major We subgroup and the V_1 subgroup with the approximate position of Gm and Inv factors on these chains

characters, which have long been known and considered controlled by allelic genes, are found in different H chain subgroups of γG globulin (22). This is illustrated in Figure 2 where the Gm (b_1, b_3, b_4) factors are all localized to proteins of the minor $V_1(\gamma_{2c})$ subgroup. Gm(a) and (x) occupy a similar position but only in proteins of the major We group.

Another finding of interest concerns the localization of Gm(f) and a new factor Gm(z) (23) to proteins of the We group, but to a different site on the H chains (24, 25). No factors in this area of the molecule have as yet been discovered for the V_1 group but these will undoubtedly be found. An extremely interesting picture is evolving concerning the inheritance of these characters which involve structures in different subgroups and different areas of each of the chains of the proteins of the different subgroups. It is already apparent that close relationships exist but the number of genetic

loci involved is not entirely clear. This work will be of undoubted significance for the understanding of the genetic mechanisms involved in antibody synthesis.

Interaction of H and L Polypeptide Chains

Another area of active investigation focuses on the antibody combining site and its relationship to the H and L chains. The Fab fragments, known to contain the antibody combining site, also contain the regions that establish the non covalent interactions between H and L chains. Antigen binds primarily to the H chain (26, 27, 28), but when the H and L chains are combined through non covalent interactions, the binding activity is enhanced (29, 30, 31). These observations may be explained by two major possibilities: a) the stable binding site may be formed by two polypeptide chains held together by non covalent interactions; b) the binding site may exist only on the H chain but the latter is stabilized by the L chains through non covalent interactions. In both of these hypotheses the non covalent interactions between the H and L chains have a relationship to the antibody binding site. A relevant question is: are the non covalent interactions non specific in that a given H chain may interact with any L chain to produce stable antibody molecules, or are the non covalent interactions specific in that a given H chain interacts with a certain L chain to produce a specific stable antibody molecule.

The early studies on separated H and L chains of γ G globulin demonstrated that L chains effectively solubilized the H chains that tend to aggregate in neutral non dissociating solvents (26). Furthermore even H and L chains from different species can form non covalent interactions to produce four chain molecules (32) indicating considerable affinity between these polypeptide chains. The specificity of the non covalent interactions between H and L chains was further studied with H and L chains from individual myeloma proteins (33). The H chains from a γ G myeloma protein can effectively combine with L chains from other myeloma proteins to form four chain molecules; the recombination is only slightly more effective with homologous chains than with polypeptide chains from two differing proteins. However in competitive experiments where the H chains had an equal chance to combine with L chains from the same γ G myeloma protein or L chains from another myeloma protein they preferentially recombined with homologous L chains. The significance of competition to bring out the preferential combination of homologous H and L chains illustrated in Table II (adapted from 33).

From the table it is apparent that the H chains from the myeloma protein Ga combined effectively with their own L chains and L chains from myeloma protein Ne when the L chains were added individually in only slight molar excess. However when both L chains were added simultaneously to the H chains then preferentially the

Table II Comparison of non competitive and competitive recombination of H and L chains

H and L chains used for recombination ¹	Per cent of L chains in reformed molecules
Ge H + Ge L	92.3
Ge H + Ne L	85.3
Ge H + Ge L Ne L	Ge 84.0 Ne 4.0
Ne H + Ne L	39.6
Ne H + Ge L	20.8
Ne H + Ne L Ge L	Ne 40.0 Ge 3.0

¹ In all mixtures 0.94×10^{-2} micromoles of H chains were used. In non competitive mixtures 1.03×10^{-2} micromoles of L chains were used. In competitive mixtures 1.09×10^{-2} micromoles of each of the L chains were used.

Ge H chains recombined with Ge L chains. Similar preferential recombination of homologous H and L chains occurs also in γ A myeloma proteins (34).

Several rabbit antibodies have been examined for preferential recombination of H and L chains. Roholt and others (35) observed that the H and L chains from antibodies to p azobenzoate and to p azobenzene arsonate recombined preferentially to form the original antibodies when in competition with nonspecific γ G L chains as determined by antigen binding activity. On the other hand the H chains from rabbit antibodies to the 2,4 dinitrophenyl determinants recombined at random with their own L chains and L chains from nonspecific γ G globulin (36). However when hapten was present during recombination in the latter experiments then the antibody H chains preferentially recombined

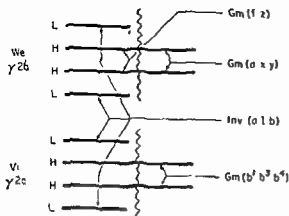


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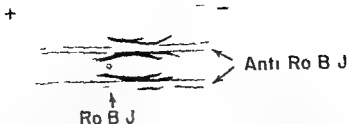


Figure 3 Immunoelectrophoresis experiment illustrating two preparations of Bence Jones protein containing considerable amounts of deficient component recognized with the homologous antiserum

proteins about 10 out of 100 peptides are present in all type L proteins while other peptides are present only in some. A number of peptides are unique for each protein as in type κ . Amino acid analyses reveal consistent differences between type κ and L Bence Jones proteins most pronounced for proline, alanine, valine, methionine and phenylalanine (39-40). A number of other interesting findings have come to light recently. The Bence Jones proteins usually appear in two different sizes in urine, one with molecular weight of about 20,000—25,000 regarded as the monomer and the other the dimer with molecular weight of about 40,000—45,000. Some of the dimers can be split by dissociating solvents such as acid, urea or guanidine. In some cases, however, reduction of one disulfide bond is necessary before dissociation can be achieved (37). In a recent report (41) the stable dimer was shown to consist of two monomers linked by a disulfide bond through the cysteine which in type κ protein is the carboxyterminal amino acid. In Bence Jones proteins type L, the corresponding cysteine is next to

the carboxy terminal end which is a serine. In a stable monomer the cysteine residue is bound to another cysteine to stabilize the sulfhydryl group. In the dissociable dimers the two monomers are linked by non covalent bonds. In addition to the monomers and dimers, there also exist smaller proteins antigenically related to the Bence Jones protein (42-43-44). Recent studies in our laboratory have shown the presence of such components in small amounts in about 30% of urines with Bence Jones protein of either type κ or L (45). They were smaller in size than the monomers and antigenically deficient compared to the Bence Jones proteins and were more readily detected by homologous antisera (Fig 3). In most cases these components were related antigenically to the variable portion of the Bence Jones protein molecule. In two cases, however, they seemed to correspond to the non variable portion.

As is the case with the other myeloma proteins, the Bence Jones proteins too have a normal counterpart. These appear both in the monomeric and the dimeric form (46) and in

with antibody L chains. The reasons for these differing observations on rabbit antibodies are not clear at this time, but might well depend on a difference of antibody response to the different haptens employed.

Studies on antibodies and myeloma proteins indicate that specificity, probably related to the primary structure, exists in the formation of non covalent bonds between different H and L chains and suggest that variability exists in the structure of the regions participating in these bonds. The extent and significance of this specificity are not clearly delineated since four chain molecules are readily formed with "inappropriate" polypeptide chains. However experiments on antibodies certainly suggest that "inappropriate" pairing of polypeptide chains does not produce effective specific antibody combining sites.

Chemical studies of Bence Jones Proteins

The greatest progress in the chemical analyses of the immunoglobulins has been made with Bence Jones proteins where the partial amino acid sequence is now available for three proteins.

Bence Jones proteins excreted in the urine of about a third of patients with multiple myeloma have been shown to be very similar or identical to the light chains of the myeloma protein in the corresponding patients (37). Thus studies of the Bence Jones proteins frequently obtainable in large amounts give valuable information about structural variation in the

light chains and can reveal possible correlations between structure and genetic determinants and mechanisms for their synthesis.

The partial amino acid sequence of three Bence Jones proteins of type K reported by Hilschmann and Craig (15) and Tilani et al (38) is therefore an important step on the way to understand the structure and variation of light chains. The sequence in the carboxy terminal half consisting of 106 amino acids of these proteins seems identical with only one exception at position 189 where two of the samples had leucine but the third one had valine. In the amino terminal half of the molecule, some parts show similar sequence when two of the proteins are compared but differ in comparison with the third e.g. the amino terminal peptide with aspartic acid at the N terminal end seems very similar in proteins Ro and Ag while the Cu protein has glutamic acid as the N terminal amino acid and other differences in the amino terminal peptide as well. Other parts of the amino terminal half seem unique for each of the proteins such as in the peptide immediately proceeding the nonvariable portion. Peptide mapping of several Bence Jones proteins of type K revealed some peptides to occur in all proteins and other peptides to be present in some but not all samples (39). A more extensive comparison has to await the complete sequence of these and several other Bence Jones proteins.

Peptide mapping of Bence Jones protein of type L (39) shows no tryptic peptides in common with type K

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crease in excretion after physical exercise (47). Thus also the Bence Jones proteins show no exceptions to the rule concerning the similarity of the "abnormal" proteins and normal immunoglobulins and antibodies. It has become abundantly clear that these proteins found in the diseases multiple myeloma and Waldenström macroglobulinemia will form the basis for an exciting chapter of biological research particularly concerning the chemical basis for the variability of the γ globulin molecule which endows it with its unique specificity as an individual antibody.

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Structural relationship between γ G- and γ M-globulin in man

By MORTEN HARBOE and JOHANNA DEVERILL

Exposure of γ G globulin to papain at pH 7.4 leads to formation of antigenically distinct fragments, the F₁b (slow) and I_c (fast) fragments (6). Structures within the F₁b fragment are also present in γ M globulin whereas the I_c fragment is specific for γ G globulin (11-18). Immunological (4, 22), biochemical (2, 3, 4, 5) and genetic (12, 16) studies have shown that the light polypeptide chains of γ G globulin which are present within the F₁b fragment are common to γ G and γ M globulin. The F₁b fragment also contains a part of the heavy chain (10, 27). It is not clear whether this part of the heavy chain the F₁d fragment is common to the two proteins. This question is of particular interest since it is generally thought that the antigen binding sites are associated with this part of the heavy chain.

The present paper describes experiments during which antigenic determinants characteristic of the I_d frag-

ment of γ G globulin were demonstrated in γ M globulin.

Materials and Methods

γ G globulin preparations Normal γ G globulin was a commercial Cohn Fr II preparation (AB Kabi Stockholm Sweden). G myeloma proteins were purified by zone electrophoresis using Pyron as supporting medium (23). Splitting with papain (papain 2 \times crystallized Worthington Biochemical Corp Freehold N.J.) and pepsin (pepsin 2 \times crystallized Worthington Biochemical Corp) was carried out as previously described (28). Tests for Gm(I) activity were used to control that digestion with pepsin was complete (15, 28). Light and heavy polypeptide chains of γ G globulin were prepared as described elsewhere (9, 17).

γ M globulin preparations γ M globulin was highly purified from sera of patients with typical Waldenström's macroglobulinaemia by a two step procedure. Depending on the physico-chemical properties of the individual macroglobulin the initial purification step was either zone electrophoresis or precipitation by dilution of serum with distilled water. The macroglobulin preparations thus obtained were further purified by density gradient ultracentrifugation (21). The protein was reduced and alkylated as described by Fleishman et al. (9).

Immunisation procedure Antisera against pepsin split γ G globulin were collected from

Demonstration of precipitin reaction between anti Fd antisera and γ M globulin

Two of the anti Fd antisera (obtained from rabbit 167 and 169) precipitated with most but not all of eleven highly purified γ M globulins in gel diffusion tests that are illustrated in figure 2. The strength of the reaction varied markedly within the group of precipitating macroglobulins even though the same concentration (1 mg/ml) of γ M globulin was used in all instances.

Nine of the anti Fd antisera failed to precipitate purified γ M globulins in similar tests.

Tests for Gm(4) in γ M globulins

The Gm(4) factor remains intact after pepsin splitting of γ G globulin (13, 19) and the determining site has been localized to the heavy chain (13). The Gm(1) and Gm(5) factors are destroyed by pepsin splitting of γ G globulin (28). Their determining sites are located on the Fc fragment of γ G globulin (12, 16) and the factors are only present on this protein (17, 12, 16, 24).

Thirteen macroglobulinaemia sera were tested for these factors in isolated γ G and γ M globulin fractions. The tests for Gm(1) and Gm(5) were performed to control the purity of the γ M globulin preparations. All γ M globulins were Gm(1, 5) whereas the γ G globulin fraction of the same sera was positive for at least one of these factors in all instances. In 13 sera the isolated γ G globulin was Gm(4) whereas the isolated γ M globulins were Gm(4).

Discussion

Rabbit antisera against human γ G globulin react with different antigenic determinants on this molecule. A major part of the antibodies usually react with antigenic determinants on the I = part of the molecule; this reaction is specific for γ G globulin. Part of the antibodies react with antigenic determinants on the light chains. These antibodies cross react with γ M globulins (4, 8, 11, 18, 22). Biochemical (2, 3, 4, 5) and genetic (12, 16) studies supplement these findings indicating that the light chains are identical in γ G and γ M globulins. The Fd fragment of human γ G globulin has been less studied because of its low antigenicity.

Pepsin splitting of human γ G globulin results in the formation of a fragment that is very similar to the Fab fragment obtained by papain splitting at pH 7.4 (25, 28). It was found that immunization of rabbits with pepsin split γ G globulin resulted in the production of potent antisera that reacted with the Fab fragment whereas no reaction with the Fc fragment was observed in the precipitation tests used in this study. After absorption with isolated light chains from normal γ G globulin the antisera still precipitated with the isolated heavy chains (γ chains). These findings indicated that the absorbed antisera reacted with the part of the heavy chain contained within the Fab fragment, the so called Fd fragment, and they are referred to as anti Fd antisera.

From the studies of the reaction of anti Fd antisera with highly purified

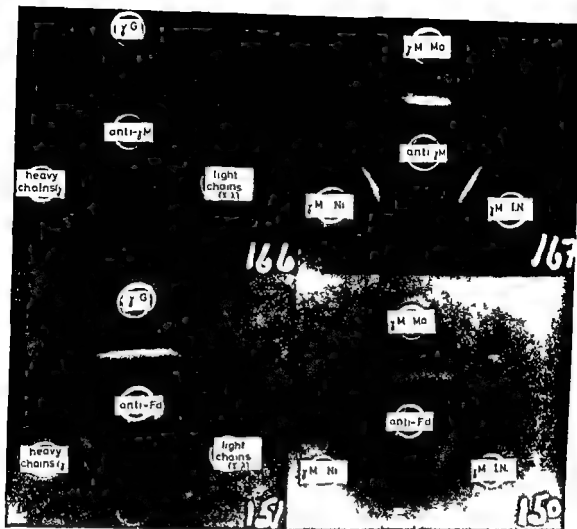


Fig 2 To show that anti Id antiserum (R167) precipitated with some but not all γ M globulins For further explanation see text

fic for the Fab fragment of γ G globulin when tested by these gel diffusion techniques All the antisera cross reacted with highly purified γ M globulin and thus appeared useful for further study of the basis of the cross reaction between γ G and γ M globulin

Reaction of the antisera with isolated polypeptide chains of γ G-globulin

All the antisera produced against pepsin split γ G globulin precipitated with

isolated light and heavy chains of γ G globulin After absorption with isolated light chains in antigen excess they still precipitated with isolated heavy chains of γ G globulin (γ chains) It was concluded that the absorbed antisera only reacted with the Fd part of the γ G globulin molecule and they are referred to as anti Fd antisera They precipitated intact γ G globulin pepsin split γ G globulin and isolated chains with reactions of identity

enzymatic splitting was not detectable in 13 purified "monoclonal" γ M globulins even though the factor was demonstrated in the γ G globulin fraction of all these sera

Acknowledgements

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fied γ M globulin, two interpretations emerge. Firstly, two of the eleven anti-Fd antisera precipitated with highly purified γ M globulins (cf. Figure 2). These findings show that antigenic determinants in the Fd fragment of γ G globulin are also present in γ M-globulin, or in other words that more than the light chains are common to these proteins. Similar findings have been made by Nussenzweig and Benicerraf (26) in studying guinea pig 7S γ_1 and 7S γ_2 globulins. These two proteins have distinct antigenic determinants in the Fc part of the molecules but share common structures both in the light chains and in the Fd fragment. Secondly, several findings indicate that the structural relationship between the Fd fragment of γ G globulin and γ M globulin is very complex. i) Nine of our anti-Fd antisera did not precipitate isolated γ M globulin. ii) Two of our anti-Fd antisera precipitated some but not all of the 'monoclonal' γ M globulins tested. Similar findings have been made by Kunkel (20). It was also evident that the strength of the precipitin lines varied considerably within the group of precipitating γ M globulins. iii) The division of "monoclonal" γ M globulins into a precipitating and a non precipitating group according to their reactions with these two anti-Fd antisera did not correspond to the antigenic heterogeneity recently described in the heavy chain of γ M globulin (17). iv) The Gm(4) factor that is intact in the Fab fragment after enzymatic splitting of γ G globulin is not present in γ M

globulin as shown above and in an independent study (13).

Antigenically distinct subgroups of the heavy chain of γ G globulin have been described recently (1, 14, 29, 30). Heterogeneity of the heavy chain of γ M globulin has been demonstrated by similar techniques (17). Further studies of these forms of heterogeneity may help in delineating the complex structural relationship between the Fd fragment of γ G globulin and γ M globulin. Such information would be of great interest since this is the part of the γ G globulin molecule that is involved in antigen binding. Little is known to date about the structure of antigen binding sites with presumed identical serological specificity in γ G and γ M globulin. Such information is needed to obtain a better understanding of the basic mechanisms involved in antibody production.

Summary

Antisera produced in rabbits against human pepsin split γ G globulin reacted with both the heavy and light polypeptide chains of γ G globulin. After absorption with isolated light chains in antigen excess they reacted only with the Fd part of this molecule.

Two out of eleven such antisera precipitated with some but not all of eleven highly purified "monoclonal" γ M globulins. These findings show that antigenic determinants in the Fd fragment of γ G globulin are also present in γ M globulin.

The Gm(4) factor that is intact in the Fab fragment of γ G globulin after

genic class of heavy and more than one intrinsic class of light polypeptide chains

It is therefore not surprising that the untypable M protein offers a challenge for further study and may often provide the first clue to an interesting and novel disorder of protein synthesis. This is strikingly illustrated by two recent experiences. In one instance failure of a protein to type for κ or λ chains led to the suspicion and subsequent description of the first patient with so called "Heavy Chain disease" a disorder associated with the production of Fc fragments (8). In another failure of a protein to react with antisera to α , γ and μ chains resulted in the discovery of a new immunoglobulin class IgD (9).

Another and possibly quite heterogeneous group of untypable myeloma proteins are the M proteins which possess one of the heavy chains but which cannot be typed for their κ and λ chain in the intact state. The first two such proteins were clearly shown to possess one type of light chain after reduction and alkylation and were thought to represent a myeloma protein with an unusual tertiary structure possibly containing hidden or inaccessible light chains (10, 11). We have recently encountered another such homogeneous protein which represents yet another mechanism.

The protein initially isolated from the serum had the following properties. It had the electrophoretic mobility of γ globulin, had a sedimentation coefficient of 3.8S at infinite dilution and belonged to the γ h (We) subtype

of γ chains. While the native protein failed to react with antisera to κ and λ chains, light chains prepared by reduction and alkylation strongly reacted with antisera to the κ . Type Reduction and alkylation yielded the expected amounts of heavy and light chains and peptide maps of these and the isolated Fc and Fab fragments produced after addition of papain were indistinguishable from other We myelomas having Type κ light chains (12).

Further studies revealed that the protein and the Fab fragment failed to precipitate with antisera to κ chains but that both inhibited the precipitation of light chains by antisera to κ chains both in quantitative precipitation tests and by causing visible shortening of the precipitin line in double diffusion analyses. Only detailed immunologic studies after completion of these initial experiments demonstrated that the material responsible for this behavior represented the products of complete spontaneous degradation which resembled in all respects the 3.5S fragments produced by papain digestion. A subsequent bleeding from this patient yielded a classical 7S γ G myeloma belonging to the We subtype and which was easily typed as belonging to the κ antigenic class.

The reason why this protein could not be readily classified becomes obvious on the basis of previous studies (13) which demonstrated that light chains may behave as univalent antigens when tested with certain antisera. Bence Jones Proteins precipitate with

The Problem of the Untypable M Protein¹

By E. C. FRANKLIN, D. FEINSTEIN and H. H. GUDENBERG

The Problem of the Untypable M Proteins

Studies of myeloma proteins and especially macroglobulins since their discovery by Professor Waldenström in 1945 (1), have yielded much information about the corresponding normal immunoglobulins which are often present in low concentrations and, consequently, difficult to isolate from normal serum.

Antigenic properties characteristic of the different polypeptide chains found in the immunoglobulins have permitted the production of a variety of antisera useful in characterizing the homogeneous protein components (M proteins) associated with a number of proliferative disorders of plasma cells and lymphocytes. Antisera specific for the α , γ and μ heavy chains generally allow classification

of such proteins into the IgA, IgG or IgM class respectively (2, 3), while antisera to κ and λ chains permit further identification of the light chains as belonging to one of the two recognized classes (4, 5). More recently antigenic analyses have resulted in the discovery of at least four subtypes of γ chains which allow even more precise typing of G myeloma proteins (6, 7). The ease of performing such immunologic analyses has resulted in the widespread use of these techniques in the classification of M proteins and it seems likely that had Professor Waldenström encountered his first patient with macroglobulinemia twenty years later he would have suspected the disorder on the basis of such immunologic tests.

Extensive studies of large numbers of M proteins have led to the conclusion that BJPs belong either to the κ or λ type and that the larger serum proteins contain one class of heavy chains and one of the light chain types. None of these proteins has been shown to contain more than one anti

¹ Supported by New York City Health 8527 and 1431 p. and by Cancer Research Research Council USPHS Grants # AM 2594 Funds of the University of California and Grant # T 356 from the American Cancer Society.

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such antisera because they normally exist as dimers, while the Fab fragment, which contains only a single light chain, fails to precipitate, but can inhibit precipitation of the appropriate BJP by combining with the antibody. In contrast, the intact IgG and the 5S pepsin F(ab)₂ fragment, each of which has two identical light chains, are precipitated by such antisera.

Based on this finding, M proteins, which can be typed for their L chain only after isolation and dimerization of their L chains but not in the intact state, may have the following structures:

1 They may be half molecules of the type produced *in vitro* by Nisonoff and Palmer (11). Such molecules could be distinguished from 7S IgG only by molecular weight determinations since they would have an amino acid composition identical to IgG but would probably not precipitate with anti light chain sera. It was initially thought that this patient may have produced such a protein.

2 They may have two identical light and two identical heavy chains but one of the light chains may be buried or otherwise inaccessible and thus unable to react with the antiserum. Such a model could be established only by exclusion if a normal molecular weight is found (10).

3 They may represent free Fab fragments, either synthesized as such or products of spontaneous degradation, as was probably the case in our patient.

4 Alternatively, such a molecule may consist of two dissimilar light chains, one of Type λ and one of Type κ . Since it has been clearly shown that λ and κ chains are not produced in the same cell, this possibility is not very likely.

If one were to rule out the fourth possibility, a choice between the other three would rest upon precise molecular weight calculations and careful antigenic analyses.

In addition to the above described possibilities, the untypable M protein, when tested with the usual antisera to heavy and light chains may represent as yet unrecognized disorders of protein synthesis. Thus, new classes of light chains and heavy chains may be discovered as was the case with IgD (9). In addition, proteins not reactive with antisera to light chains may represent "heavy chain" (Fc fragment) disease related to the α and μ chains. Finally if the six chain model of γ globulin shall prove to be correct, Fd fragments only may be produced by certain patients and these would not be expected to react with antisera to light chains or the Fc fragment determinants of heavy chains. Thus, it is obvious that careful study of all untypable M proteins is warranted and that such studies may uncover examples of hitherto undescribed disorders of protein synthesis.

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Biological significance of exocrine gamma-A-immunoglobulin

By JOSEPH F HEREMANS, PAUL A CRABBE and PIERRE L VASSON

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University of Louvain (Leuven) Belgium

Gamma-A-immunoglobulin (also called IgA gamma 1 λ or beta 2 λ) is a molecular variety of antibody active proteins, which was first isolated and characterized by one of the present writers during work on myeloma proteins at Professor Waldenström's laboratory in the summer of the year 1957 (18). It represents about 20 per cent of the total circulating immunoglobulins, and investigations from various sides have demonstrated that it is the carrier of a considerable spectrum of antibody activities (rev. cf. ref. 17). In this respect it seems not inferior to the more abundant form of immunoglobulins *viz.* gamma G (formerly called 7S gamma). Therefore the question may be asked why the organism should find it advantageous to multiply the molecular species of effecting identical antigen combining tasks, in other words why gamma A should exist at all.

To discuss this point it may be profitable to consider the following facts and concepts which have emerged out of old and recent research: (1) the

distribution of gamma A in various biological fluids, and (2) the concept of *exocrine antibodies*.

(1) *Distribution of gamma A immunoglobulins in various biological fluids*

Although the concentration of gamma A in normal plasma is only about one quarter of that of gamma G, the situation is very different in all biological fluids that are not mere transudates of the plasma. Thus the concentration of gamma A much exceeds that of gamma G in milk and colostrum (14), saliva (22, 25, 33), tears (5), nasal secretions (29), bronchial secretions (21, 24, 28), as well as in secretions from villous tumours of the rectum (27). Notwithstanding the fact that large amounts of plasma proteins continuously leak into the digestive tract, the gamma A: gamma G ratio in the fluid obtained by intestinal aspiration is higher than the corresponding ratio in the serum (5), and the same is observed in bile (5). Some kind of lo-

cal production of gamma Λ must therefore be assumed at all such sites unless one is willing to admit a preferential transfer of gamma Λ from the plasma

The latter question now appears to be solved. The gamma Λ present in exocrine secretions is obviously largely generated in the glands or surfaces responsible for their production and not derived from the plasma. This is demonstrated by the failure of intravenously injected ¹²⁵I labelled gamma Λ to appear in the parotid fluid (32) as well as by the fact that salivary glands (20, 32), mammary glands (20) and intestinal mucosa (2) incorporate labelled amino acids into gamma Λ immunoglobulin. The connective tissue stroma of all these structures is richly endowed with plasma cells, the accepted sources of immune globulins. For several years our group has been engaged in an immunohistochemical study of those mucosal and glandular plasma cells with the aim of verifying the nature of the immunoglobulins they contain. It has thus been shown that the lamina propria of the duodenum and upper part of the jejunum numbers 300 000 gamma Λ containing cells per cubic mm, on the average, against only 15 000 gamma Γ containing cells and 51 000 gamma Λ containing cells per cubic mm (6, 7). A typical field of lamina propria surrounding the crypts of Lieberkuhn and displaying a wealth of plasma cells filled with gamma Λ is illustrated in Fig. 1. Similar findings have been made on other sections of the gut (9)



Fig. 1 Plasma cells containing gamma Λ immunoglobulin in a normal human intestinal mucosa. Frozen section through the crypts of Lieberkuhn of a duodenal biopsy, stained with fluorescein labelled antiserum against gamma Λ immunoglobulin. The plasma cells form dense sheaths around the crypts.

as well as on the stroma of salivary glands and of the bronchial mucosa.

Whereas the bulk of gamma Λ present in secretions is thus demonstrably independent from any supply by the plasma, it may conversely be asked to what extent such local production of gamma Λ may contribute to the circulating pool of this protein. Indirect evidence pleads against the assumption that such a contribution from exocrine sources would be significant. The exocrine type of gamma Λ displays one significant physicochemical difference with respect to its counterpart in the plasma, viz. its high molecular weight. This has been demonstrated for the gamma Λ from milk and saliva (32) as well as for that from bronchial secretions (26). In all such secretions the bulk of the gamma Λ sediments in the ultracentrifuge as a heterogeneous population of components, chief of which are those with

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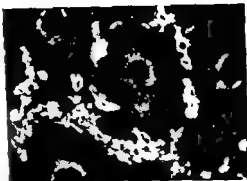


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Whereas the bulk of gamma λ present in secretions is thus demonstrably independent from any supply by the plasma it may conversely be asked to what extent such local production of gamma λ may contribute to the circulating pool of this protein. Indirect evidence pleads against the assumption that such a contribution from exocrine sources would be significant. The exocrine type of gamma λ displays one significant physicochemical difference with respect to its counterpart in the plasma viz. its high molecular weight. This has been demonstrated for the gamma λ from milk and saliva (32) as well as for that from bronchial secretions (26). In all such secretions the bulk of the gamma λ sediments in the ultracentrifuge as a heterogeneous population of components chief of which are those with

sedimentation rates of 9S and 11S. In contrast, the gamma A immunoglobulin from normal human serum is mainly present under the form of a monomer having a sedimentation coefficient of about 7S. A small proportion of polymers, accounting for 10—15 per cent of the total (15, 16) is also present in the serum, and does not seem to be an artifactual aggregation product ascribable to the method of isolation. Therefore, if part of the exocrine gamma-A is assumed to reach the bloodstream, it can hardly account for more than one tenth, possibly less, of the total circulating pool of gamma A.

(2) *The concept of exocrine antibodies*

In 1922 attention was drawn by Davies (10) on the fact that stools from patients with bacillary dysentery often contained agglutinating antibodies against *Shigella*, in the absence of demonstrable agglutinating activity in the serum. Experimental work with cholera vaccine, administered by way of the mouth or the stomach in guinea pigs (1) and the human (12, 13) has led to the concept that a particular class of antibodies, called "coproantibodies" are synthesized by the digestive tract upon local contact with the antigen. Such antibodies appear to have distinct biological properties: their titers in the secretion reach an earlier and higher maximum but wane faster than the corresponding titers of circulating antibodies directed against the same antigen. Antibodies

with similar behaviour have also been raised in the human female genital tract upon intravaginal application of an antigen (31), and the local production of antibodies in the udder of lactating cows upon intramammary introduction of antigens is an established knowledge in veterinary medicine. We may therefore assume that the above described properties are not restricted to "copro antibodies" or "mucosal antibodies", but are characteristic for exocrine immunoglobulins in general.

In view of the unique predominance of gamma A immunoglobulin in all types of exocrine secretions, and considering that the overwhelming majority of the subepithelial plasma cell population is obviously engaged in the production of precisely this type of immunoglobulin, one cannot escape the conclusion that exocrine gamma A immunoglobulin and exocrine (mucosal) antibody must be one and the same thing.

If this equation has any meaning we may now turn to the question as to why a special type of immunoglobulin should be required to serve as an exocrine antibody. Perhaps the answer is twofold.

In the first place there is one physicochemical property of gamma A that seems to make it eminently suitable for such a role. To resume a term coined by Burnet (3) the variety of local antibody here discussed should be viewed as an "antiseptic paint". Now paints are by definition endowed not only with coating but also with adhesive properties. This is precisely the case with

gamma A, which has an unusual tendency to form complexes with the most diverse kinds of other proteins (10). One may even guess that the same property allows it to act as a skin sensitizing antibody i.e. as a carrier of allergic reactivity (11, 19, 23).

There is however another aspect to the point. The antibodies synthesized by the lamprey, a form of *Chordata* even more primitive than the earliest vertebrates, appear to have molecular weights intermediate between those of gamma G (7.5) and gamma M (19.5) antibodies but falling in the range of the polymeric forms of gamma A encountered in exocrine secretions (1). Now lampreys do not possess any well defined lymphoid organs such as lymph nodes: their intestinal mucosa however rests on a connective tissue stroma whose lymphoid cell population is much reminiscent of that in man. It would not be surprising therefore if gamma A eventually turned out to be an ancestral type of immunoglobulin formerly exclusively associated and presently still largely restricted to production sites close to the epithelial surfaces by which the organism establishes contact with the aquatic environment of the outside world.

If the latter assumption proves true then gamma A might be the living fossil among the immunoglobulins and evolution may be expected to discard it sooner or later. Perhaps this is what is actually being experimented already in those extremely rare individuals who in spite of a perfect health



Fig. 2 Duodenal biopsy from a patient with idiopathic steatorrhoea associated with a severe deficiency of gamma A immunoglobulin in the serum and in all secretions. Only a few fluorescent plasma cells are visible in this section which has been stained with fluorescein labelled antiserum against gamma A.

seem to be entirely devoid of gamma A immunoglobulins (30). Still some aspects of the function of gamma A as exocrine antibody may nevertheless remain of importance. This is suggested by the discovery of a curious syndrome (8) in which idiopathic steatorrhoea is associated with an apparently very important lack of gamma A immunoglobulins in the serum, the exocrine secretions and the plasma cells of the intestinal wall (Fig. 2).

Summary

Gamma A immunoglobulin is found in much higher relative concentrations in all exocrine secretions than in the serum.

Two interpretations are given for this observation. It would seem that gamma A is the material substrate for

sedimentation rates of 9 S and 11 S. In contrast, the gamma A immunoglobulin from normal human serum is mainly present under the form of a monomer, having a sedimentation coefficient of about 7 S. A small proportion of polymers, accounting for 10—15 per cent of the total (15, 16) is also present in the serum, and does not seem to be an artifactual aggregation product ascribable to the method of isolation. Therefore, if part of the exocrine gamma-A is assumed to reach the bloodstream, it can hardly account for more than one tenth, possibly less, of the total circulating pool of gamma-A.

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Sedimentation Constants of IgG and IgD Myeloma Proteins Compared with those of Normal IgG

BY ULLA BRITT HANSSON, C. H. LAURELL and R. BACHMANN

In 1963 Rowe and Fahey (9) described the characteristics of the new class of immunoglobulins IgD. One isolated IgD myeloma protein was found to have a sedimentation constant ($s_{20,w}$) of 7.0 against 6.6 for normal IgG. Of our series of 1300 sera with M components, four were identified as IgD. Three of these samples were still large enough to allow isolation of the M component and estimation of the sedimentation constants. Their constants were lower than or the same as our constants for normal IgG. We therefore compared the sedimentation constants of some isolated M components of types IgG and IgD with those of some isolated individual normal immunoglobulins.

Material and methods

Frozen sera with M components of type IgD (number 510, 793 and 104) and of type IgG (number 163, 909, 8, 34 and 226) stored at -15°C for one to several years.
Fresh serum with M components of type IgG (number 1311).
Fresh normal individual sera (number 1 to 8).

Paper electrophoresis was run in barbital buffer 0.05 M containing 2 mmol CaCl₂.

Isolation of M component (IgG and IgD) and of normal IgG. For the studies 1123 to 1230 g IgG (IgD) was used. One part serum was diluted with three parts 0.9% NaCl and ammonium sulphate was added to a final concentration of 1.84 M with stirring at room temperature. The pH was adjusted with ammonia to 7. After two hours at $+4^{\circ}\text{C}$ the precipitate was centrifuged down, washed, resuspended three times in ammonium sulphate (1.84 M) at pH 7 and $+4^{\circ}\text{C}$ and dissolved. The sample was dialysed first for at least 2 hours against running tap water and then over night against 0.9% NaCl and finally for at least 3 hours against 0.02 M phosphate buffer pH 8.0. The material was fractionated on a DEAE column using a salt gradient with increasing ionic strength and decreasing pH (400 ml 0.02 M phosphate buffer pH 8.0 + 400 ml 0.2 M phosphate buffer pH 6). The eluate containing the desired protein was concentrated to a volume of 4 ml by combined ultrafiltration and dialysis against 0.05 M phosphate buffer pH 7.0 made 0.5 M in respect of NaCl. This solution (roughly 4 per cent) was applied to the top of a column filled with Sephadex G 200 equilibrated with the same buffer. The filtration was started in the night by an electric switch watch at such a time that the desired fraction appeared in the effluent in the morning of the day that the centrifugation series of each component in different dilutions was to be run.

what has long been known as mucosal antibodies. The special tendency of this protein to combine with other proteins would make it qualified for the rôle of an antiseptic paint. It is also possible, however, that gamma A merely represents an ancestral form of immunoglobulin, initially exclusively associated with the lymphoid tissue from the connective structures supporting the surfaces by which the organism makes contact with the antigens from the outside world.

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Sedimentation Constants of IgG and IgD Myeloma Proteins Compared with those of Normal IgG

By ULLA BRITT HANSSON, C. B. LAURELL and R. BACHMANN

In 1963 Rowe and Fahey (9) described the characteristics of the new class of immunoglobulins IgD. One isolated IgD myeloma protein was found to have a sedimentation constant ($s_{20,w}$) of 7.0 against 6.6 for normal IgG. Of our series of 1300 sera with M-components, four were identified as IgD. Three of these samples were still large enough to allow isolation of the M-component and estimation of the sedimentation constants. Their constants were lower than or the same as our constants for normal IgG. We therefore compared the sedimentation constants of some isolated M-components of types IgG and IgD with those of some isolated individual normal immunoglobulins.

Material and methods

Freeze sera with M-components of type IgD (number 310, 33 and 304) and of type IgG (number 163, 309, 893 and 276) stored at -15°C for one to several years. Free serum with M-components of type IgG (number 1711) and individual normal sera (number 1) to 8.

Paper electrophoresis was run in barbitol buffer 0.05 M containing 2 mmol CaCl₂.

Isolation of M-component (IgG and IgD) and of normal IgG. For the studies 023 to 030 g IgG (IgD) was used. One part serum was diluted with three parts 0.9% NaCl and ammonium sulphate was added to a final concentration of 1.84 M with stirring at room temperature. The pH was adjusted with ammonia to 7. After two hours at $+4^{\circ}\text{C}$ the precipitate was centrifuged down, washed, resuspended three times in ammonium sulphate (1.84 M) at pH 7 and $+4^{\circ}\text{C}$ and dissolved. The sample was dialysed first for at least 2 hours against running tap water and then overnight against 0.9% NaCl and finally for at least 24 hours against 0.02 M phosphate buffer pH 8.0. The material was fractionated on a DEAE column using a salt gradient with increasing ionic strength and decreasing pH (400 ml 0.02 M phosphate buffer pH 8.0 + 400 ml 0.2 M phosphate buffer pH 6). The eluate containing the desired protein was concentrated to a volume of 4 ml by combined ultrafiltration and dialysis against 0.05 M phosphate buffer pH 8.0 made 0.5 M in respect of NaCl. This solution (roughly 4 per cent) was applied to the top of a column filled with Sephadex G 200 equilibrated with the same buffer. The filtration was started in the night by an electric switch watch at such a time that the desired fraction appeared in the effluent in the morning of the day that the centrifugation series of each component in different dilutions was to be run.



Fig 1 Agar gel electrophoretic pattern of the ultracentrifuged M components and normal γ globulin fractions (For serial number see Table I) No 1311 represents preparation one. The second appeared identical.

Agar gel electrophoresis was run on 1% agarose in 0.1 M barbital buffer pH 8.4.

Ultracentrifugation was done at 59760 rpm in a Spinco Analytical Ultracentrifuge Model L with Schlieren optics. The temperature was 20°C and the buffer used was the same as that in the Sephadex column. Each sample was run at three concentrations between 0.2 g per 100 ml to 1 g per 100 ml. At each concentration five pictures 8 minute intervals were taken.

Evaluation of the plates was done with two dimensional microcomparator (Lestz).

Calculation of sedimentation constants. The differences $(\ln x_2 - \ln x_1)^2$ were plotted (y axis) against the concentration (x axis). The straight line through the twelve points was calculated by the method of least squares where the sum of squares of the vertical differences was reduced to a minimum. The intersection of this line with the y axis gave the difference at an infinite dilution in buffer at 20°C. The s value at infinite dilution in buffer at 20°C was calculated according to Schachman (11) and the s value in water at

¹ x_2 and x_1 are the distances from the centre of rotation to the maxima of peaks in cm at times t_2 and t_1 .

20°C according to Jahneke and Scholtan (4). The standard error of a single determination was calculated and expressed as a percentage of the sedimentation constant.

Immunologic identification. M components 510-793 were classified by Dr J Fahey as IgD. M component 510 was purified and used to immunize rabbit. The antiserum obtained was anti IgD specific after absorption. Case 1074 was revealed with this antiserum. Specific antisera against the immunoglobulin chains gamma, kappa and lambda were available.

Results

The results of agarose gel electrophoresis of the purified immunoglobulins used are shown in Fig 1. The purified IgD M component No 1074 was contaminated with IgG. The electrophoretic mobility of the IgD-M component (β_2 - γ_1) agrees with the mobility given by Rowe and Fahey (10) for normal IgD globulins.

The sedimentation constants calculated for the 3 IgD and the 5 IgG M components are compared with estimated constants of individual purified polyclonal IgG fractions (Table I). In the assessment of the reproducibility of the preparation, the run and the evaluation, the constant was estimated three times with the sample with serial No 3 and twice with sample 1311. The results obtained with the third preparation of No 3 showed that the apparatus was unaltered during the time used for the experiments. Serial sample No 1 was also analysed twice but on the same day and with the same preparation to test the reproducibility of the run and the evaluation. The standard errors expressed as per

Table I

Ultra centrifugal run No	Serial No	$s_{0,20} w^1$	SD ²	Globulin type	Paper electrophoretic mobility	Light chain type	Gm
56 57 58	510	6.54	0.9	M comp IgD	$\beta-\gamma_1$	L	—
59 60 61	793	6.76	0.8		"	L	a-f-b-
126 127 128	1014	6.19	1.0		γ_1	L	a-f-b-
109 110 111	165	6.81	0.4	M comp IgG	γ	h	a-f-b-
113 114 115	909	6.77	0.6		γ_3	h	a-f-b-
116 117 118	8	6.46	0.6		$\gamma_1-\gamma_2$	h	a-f-b-
120 121 122	94	6.91	0.4	"	γ_1	I	a-f+b-
123 124 125	226	6.72	0.5		$\beta-\gamma_1$	h	a-f-b-
129 130 131	1311	6.82	0.5		γ_1	h	a-f-b-
142 143 144	1311	6.78	1.0	Normal IgG	$\gamma_1-\gamma_3$	—	a+f+b+
3 4 75	1	7.01	0.8		"	—	a-f+b+
10 80 81	2	6.91	0.6		"	—	a-f+b+
11 77 78	3	6.88	1.0	"	"	—	a-f+b+
51 92 93	3	6.89	0.6		"	—	—
141 132 143	3	6.90	0.5		"	—	—
94 95 96	4	6.79	0.5	"	"	—	a+f+b+
79 100 101	4	6.78	0.3		"	—	—
107 103 104	5	6.77	0.7		"	—	a+f+b+
137 140 141	6	6.6	0.6	"	"	—	a+f+b+
145 146 147	7	6.81	0.6		"	—	a+f+b+
148 149 150	8	6.83	0.7		"	—	a+f+b+

¹ Sed const. at infinite dilution in water at 20°C

² Standard error expressed as percentage of the sedimentation constant

centage of the sedimentation constants varied between 0.3 and 1.0 (Table I). The values found for IgD M components varied between 6.19 and 6.76 and for IgG M components between 6.46 and 6.91. The IgD M components represent the lowest value found. The normal individual IgG preparations varied between 6.76 and 7.01. The light chain type of the M components are given in Table I. The three IgD M components had a light chain of type L. Of the six M components of type IgG five had a light chain of type h and one a light chain of type L. The Gm types were estimated by Dr H

Grubb on the individual normal and pathological IgG globulins and are given in Table I. All M components were Gm (a-f-b-) except No. 94 that was Gm (i-f+b-). Of the normal immunoglobulins 6 were Gm (a+f+b+) and 2 were (a-f+b+).

Discussion

The molecular polymorphism of IgG is well known from e.g. variation in charge in Gm type and content of kappa or lambda chains if we disregard the difference in antibody specificity. The corresponding M compo

nents are further known to vary *eg* in N-terminal amino acids and in peptide patterns on fingerprinting. It is evident from the literature that the S_{20} w -value found varied considerably among M components of 7S- γ -globulin type. But it is not possible to decide to what extent this depends on the measuring technique and the impurity of the preparation of the component since, in general, no data are available for the immunoglobulin type, degree of purity, ultracentrifugal and electrophoretic homogeneity. The findings presented here indicate that freshly prepared M components of IgG and of IgD type vary in S rate outside the error of the analytical method. Our IgD components gave S rates lower than, or equal to those of the IgG globulin (normal and pathological) in contrast to the single observation reported by Rowe and Fahey (9). Also the sedimentation constants for individual normal IgG varied outside the error of the analytical method. It has to be elucidated whether this variation is correlated with any of the variations observed in the properties of the light or heavy chains of the immunoglobulins (1, 2, 3, 4, 5, 6, 8). No link was found with the Gm system.

Summary

The sedimentation constants for normal IgG globulins varied (6.76–7.04) outside the error of the analytical method. Six IgG M components varied between 6.16 and 6.91 and 3 IgD M components gave 6.54, 6.76 and 6.19

Acknowledgement

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Ultracentrifugal Plasma Protein Pattern and Age in Healthy Men

By L. L. BÜTTIGER, L. A. CARLSON and S. HEDMAN

The pioneering work of Pedersen (8) in the 1940's made it possible to apply the analytical ultracentrifuge to the study of the composition of the serum proteins. One of the major advantages of this method is that it permits estimation of macroglobulins present in plasma. Waldenström was the first to introduce this technique into clinical medicine when he demonstrated the presence in serum of high amounts of macroglobulins in the syndrome later given his name (10, 11).

The interest in macroglobulins in Waldenström's disease and related disorders (cf. 3) and in connection with the rheumatoid factor and various antibodies has been steadily increasing. Nevertheless there are only few studies on the values for macroglobulins in healthy subjects (cf. 5). No material has been studied with regard to the possible influence of age. We therefore found it of value to establish normal levels in serum for macroglobulins in healthy individuals of various ages.

Methods

Material

Sera from healthy men in different age groups were analyzed. The men had initially been randomly sampled from the population of Stockholm and had all taken part in a complete physical and laboratory health survey in 1954-55 (3) and again in 1958-59 (1). The criteria used to select the healthy men were described previously (1, 3).

In 1961 a group of men who were accepted as healthy in 1958-59 and whose health had not changed since were called upon to participate in the present study.

The following laboratory tests were done: Hemoglobin, ESR and urinalysis for glucose and protein. The previously defined "normal" limits were used (1). Only two subjects were excluded, the reason being an ESR of 22 and 27 mm/hour respectively.

Analytical methods

Blood was withdrawn in the morning after fasting over night by venipuncture and allowed to clot at room temperature. Serum was separated off by centrifugation within 1 to 2 hours and its protein concentration determined with the biuret method (13). The serum was diluted with buffer (NaCl 0.17 M, NaH_2PO_4 0.03 M, NaHCO_3 0.02 M) to a protein concentration of 1 g per 100 ml. The ana-

lysis were done in the Spinco Model L ultracentrifuge with the analytical rotor D and the 12 mm analytical cell at a speed of 59 780 r.p.m. The runs were made at a rotor temperature of 20°C. In all sera only three components were visible. They have been called A, G and M component (cf 5) in order of increasing sedimentation coefficient. The relative amounts of the components were estimated by simple planimetry and the sedimentation coefficients of the components were calculated and adjusted to 20°C S_{20w} and the viscosity of water (5). No Johnston Ogston correction for superimposed gradients of slow components at faster boundaries was applied and the sedimentation rates obtained were not extrapolated to zero protein concentration (6).

Results

The concentration of total plasma proteins did not differ in the four age classes as seen in Table I. The mean

plasma protein concentration in the whole material was 7.8 g per 100 ml.

Table I also shows that the percentage composition of the plasma proteins with regard to the three main ultracentrifugal fractions, A, G and M components, did not change with increasing age. The mean value for the A component was 76.7 per cent and for the G and M components 18.3 and 5.0 per cent.

As there were no differences in either the total amount or in the percentage composition of the plasma proteins all values were pooled and the frequency distribution of the A, G and M components studied. Figure 1 shows the range of the values for these components and also indicates that there is no major deviation from a normal distribution.

Table I Serum protein concentration, relative distribution and S_{20w} value of the A, G and M components in the different age groups

Age group years		n	Serum proteins g/100 ml	A component		G component		M component	
				per cent ¹	S_{20w}	per cent ¹	S_{20w}	per cent ¹	S_{20w}
30—39	M ± SEM	18	7.8 ± 0.1	77.5 ± 0.9	40 ± 0.0	17.7 ± 0.8	6.8 ± 0.1	4.7 ± 0.5	17.0 ± 0.1
	S		0.5	3.7	0.1	3.5	0.2	2.0	0.5
40—49	M ± SEM	11	7.6 ± 0.1	77.5 ± 1.6	40 ± 0.0	17.9 ± 1.4	6.6 ± 0.1	4.6 ± 0.3	16.8 ± 0.2
	S		0.4	4.8	0.1	4.3	0.2	0.8	0.5
50—59	M ± SEM	15	7.9 ± 0.1	75.1 ± 1.3	40 ± 0.0	19.1 ± 1.1	6.6 ± 0.1	5.8 ± 0.5	16.8 ± 0.1
	S		0.4	5.1	0.1	4.1	0.3	1.9	0.5
60—71	M ± SEM	4	7.9 ± 0.2	76.5 ± 0.7	40 ± 0.1	19.2 ± 0.7	6.4 ± 0.0 ²	4.3 ± 0.2	16.5 ± 0.3
	S		0.5	1.3	0.1	1.4	0.1	0.5	0.6
30—71	M ± SEM	46	7.8 ± 0.1	76.7 ± 0.6	40 ± 0.0 ³	18.3 ± 0.5	6.6 ± 0.0 ³	5.0 ± 0.3	16.9 ± 0.1 ³
	S		0.4	4.3	0.1	3.6	0.3	1.7	0.5

M = mean value SEM = Standard error of the mean S = Standard deviation n = number
 S_{20w} = Sedimentation coefficient at the total protein concentration of 1 g per 100 ml adjusted to 20°C and the viscosity of water

¹ = Per cent of total amount

² = 3 subjects

³ = 45 subjects

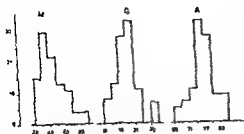


Figure 1 Frequency distribution of the three ultracentrifugal components in the whole material

The sedimentation coefficient for the three fractions did not vary with age (Table I). The mean value for the Λ component was 40 and for the G and M components 66 and 169 respectively.

Discussion

The ratio albumin/globulin in serum determined with different methods such as precipitation and electrophoresis has been reported to decline with age in man (for review see 7).

In this material on the other hand the Λ component which contains the serum albumin did not change from 30 to 70 years. The Λ component comprises however not only serum albumin but also other serum proteins such as the Λ component of Pedersen (8) but by far the major part is accounted for by the albumin. It is not likely that increases in the minor non albumin constituents of the Λ component could have contributed to any major extent to our finding of a constant level of the Λ component by masking a decrease in the concentration of albumin. The Λ component for

instance which is lipoprotein and constitutes less than 10 per cent of the Λ component cannot have increased more than a few per cent from 30 to 70 years as evidenced by lipid and lipoprotein analysis of this material (1). It appears more likely that the differences are due to the composition of the materials studied. It was indeed pointed out by Rafsky et al (9) that in their material of 31 "normal" old subjects chronic degenerative diseases may have been present. Major diseases of that kind have most likely been excluded from our material of clinically healthy men. In accordance with this Woodford Williams et al have recently reported that age has no apparent effect on serum protein levels in subjects considered disease free (14). They separated the serum proteins with electrophoresis and found that immobility and various disease processes and not age were responsible for observed decreases in the albumin/globulin ratio.

Few materials are available on the ultracentrifugal composition of plasma proteins. Values for 20 "normal" sera age not given analysed under conditions which separated the Λ component from the Λ component were given by Jahnke and Scholtan (5) as follows: Λ component 56, M component 77.9, G component 13.9 and Λ component 2.6 per cent of total protein concentration. Wallenius et al (12) who analysed lipid free sera found other values with lower concentration of the Λ component and higher of the G and M components. He also analysed

ed sera after zone electrophoretical separation and stressed the fact that the M component is composed of approximately the same amounts of α_2 and gamma globulins. In four normal men (age 25—41, criteria for selection not given) Eriksen (2), analysing sera diluted 5—7 times with M/10 NaCl, found a mean value for the M component of 0.26 g/100 ml while our figure was 0.39 g/100 ml. A direct comparison between these previous and the present results is not possible as the conditions of centrifugation (protein concentration, density, pH, temperature) were different in these studies, which may have a significant influence on the quantitative results.

Summary

The serum protein concentration and composition analysed in the analytical ultracentrifuge has been studied in 46 clinically healthy and randomly selected men from 30 to 70 years. The total serum protein concentration and the percentage amount of the A, G and M-components remained constant over this age span.

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The Frequency of Pronounced Polyclonal Hypergamma-globulinaemia in a Random Population

By U. AXELSSON and J. HALLIN

On electrophoresis of serum an increased gamma globulin fraction is sometimes seen as a diffuse and sometimes as a more localized increase. The latter type is seen as a sharp peak in moving boundary electrophoresis and as a narrow band on paper electrophoresis. Referring to Burnet's clonal selection theory (2) Waldenström (13) called this type of increase monoclonal hypergammaglobulinaemia. This pattern is seen mostly in myelomatosis and macroglobulinaemia (Waldenström) but is also found in other conditions as described by Waldenström (12, 14, 15).

Diffuse or polyclonal hypergammaglobulinaemia is most pronounced and most common in systemic lupus and cirrhosis of the liver and is sometimes seen in rheumatoid arthritis, chronic sinusadenitis, thyroiditis and discoid lupus, i.e. conditions more or less closely related to each other and to the two first mentioned diseases.

1. Protracted infections are often attended by moderate polyclonal hypergammaglobulinaemia. Such an in-

creased gamma globulin fraction has also been found in apparently healthy persons (9). The heredity of polyclonal hypergammaglobulinaemia has been studied by Leonhardt (6).

From 1963 to 1965 a mass health control sponsored by the Swedish National Board of Health was carried out in a district of Sweden. At the suggestion of Professor Waldenström this investigation was extended to include paper electrophoresis of sera from the inhabitants in one part of the district mainly to estimate the frequency of monoclonal hypergammaglobulinaemia in a random population. A paper dealing with the result of this investigation is in press (1). This paper is concerned with an estimate of the frequency of pronounced (≥ 2.0 g/100 ml) polyclonal hypergammaglobulinaemia in the population under discussion.

Material and methods

12 persons above 20 years of age were invited to take part in the health control. During a 3 month period we obtained sera from

Table 1 Data concerning 12 cases with polyclonal hypergammaglobulinaemia

Case	Sex	Age	Alb	γ	ESR mm/h	Waller Rose	FIIA	A \ N F	Diagnosis
1 (4471)	F	43	5.6	2.2		128	2560	neg	No subjective symptoms
2 (75)	M	73	5.1	2.3	113	206	2060	neg	No subjective symptoms
3 (88)	F	52	5.4	2.0	40	128	2060	neg	Cardiosclerosis
4 (2482)	F	56	4.6	2.3	25	neg	neg	neg	Bronchial asthma cancer
5 (5422)	F	33	5.1	2.0	12	64	2060	neg	Diffuse joint pain
6 (100)	F	67	3.7	2.7	80	64	2560	neg	Rheumatoid arthritis
7 (5519)	F	60	5.2	2.3	100	128	2560	neg	Rheumatoid arthritis
8 (8300)	F	62	4.5	2.3	80	neg	neg	++	Rheumatoid arthritis
9 (449)	F	62	4.0	4.7	65	neg	80	++	Chronic hepatitis
10 (7108)	F	57	4.8	2.3	41	256	2560	neg	Cholangiolitis — cirrhosis
11 (6150)	F	40	4.4	3.4	128	neg	640	neg	Hyperglobulinaemic purpura
12 (6511)	F	46	5.1	2.9	92	32	neg	neg	Hyperglobulinaemic purpura

Alb γ albumin and gammaglobulin fractions (g/100 ml) on paper electrophoresis

Waller Rose sensitized sheep cell test

FIIA FII acryl particle fixation test

A \ N F antinuclear factors

6995 persons i.e. 70% of the population above 20

All sera were studied by paper electrophoresis (4). The strips were inspected visually for polyclonal hypergammaglobulinaemia and the protein fractions of hyperglobulinaemic sera were studied quantitatively.

At the Department of Clinical Bacteriology in Malmö (Head S. Winblad MD) antinuclear factors (A \ N F) were demonstrated by the method used by Leonhardt (6). The heterophile absorbed sheep cell test (Waller Rose) and FII acryl particle fixation test (FIIA) were done according to Winblad (17).

We tried to obtain as much pertinent information as possible about the subjects with pronounced hypergammaglobulinaemia. Some of them were examined clinically because of abnormal findings in the blood chemistry at the screening of the mass health control. Concerning the others information was obtained from hospitals or from the subjects themselves.

Results

Polyclonal hypergammaglobulinaemia was suspected on visual inspection of

the paper electrophoretic strips of 28 of 6995 sera studied. The concentration (g/100 ml) was less than 1.5 in 4, 1.5 or more in 12 and 2.0 or more in 12 sera.

Table I gives data about the 12 last mentioned cases. One subject was a male aged 73. The mean age was 54 years.

The highest gamma globulin value found was 4.7 g/100 ml. Albuminopenia (<4.2 g/100 ml) was found in two cases. The ESR usually reached high levels and was normal in only one case.

The Waller Rose test and/or the FIIA test usually both were clearly positive in 9 cases. A \ N F were found in case 8 where rheumatoid factors were not found and in case 9 where only the FIIA test was positive.

One woman (case 1) had never felt well. The only man (case 2) in the

series was an old farmer who had been admitted to hospital because of mild anaemia and a high ESR. No signs of malignant disease had been found. Liver function had not been studied. One year later, at the time of the health control his general condition was good.

One woman (case 3) had been receiving medical treatment for several years for atrial fibrillation and cardiac insufficiency. She was known to have had a moderately high ESR for years.

One woman (case 4) had had allergic rhinitis, asthma and drug allergy. She had also been operated upon for cancer of the vulva.

One young woman (case 5) had intermittent joint pains but no signs of arthritis. Three women (cases 6, 7 and 8) had rheumatoid arthritis. In one (case 7) of these the joint symptoms had been initiated by drug allergy and parotid swelling.

One woman (case 9) had had diabetes for 20 years. One year before our examination she had been treated in hospital where jaundice, a high transaminase level and extremely high gamma globulin concentration (>6 g/100 ml) had been found. Her condition was conceived by us as chronic hepatitis. Five years before the health control one subject (case 10) had undergone appendectomy and a carcinoma had been diagnosed histologically. Three years later the ESR had been high and a tumour had been suspected. Liparotomy had revealed nothing abnormal but histological examination of a liver biopsy speci-

men had shown cholangiolitis and moderately advanced cirrhosis.

Cases 11 and 12, aged 40 and 46, had purpura of the legs. The former had had the condition for two years and the legs were moderately discoloured. The latter had had this condition for 20 years and her legs were strongly discoloured up to the groins. The bleedings were more pronounced when she had been standing for a long time and during thunderstorms. Both patients otherwise felt well and were not at all incapacitated by their symptoms.

Discussion

In order to get a complete series in spite of the rough screening method only cases with pronounced (≥ 20 g/100 ml) hypergammaglobulinaemia were accepted in the present investigation.

The frequency was about 1 per 600 persons above 20 years of age. Hyperglobulinaemia of this level is often seen in association with various diseases such as systemic lupus and cirrhosis of the liver. It is very likely that some persons with such diseases did not take part in the health control because they were already under medical care. The frequency found must therefore be regarded as somewhat too low. For comparison it might be mentioned that two persons with monoclonal hypergammaglobulinaemia of 20 g/100 ml or more were found.

The mean age of the subjects (53 years) did not differ from that of the

primary series (51 years) The female dominance is in good agreement with the findings in series with such diseases as systemic lupus (8), non alcoholic liver cirrhosis (3) and rheumatoid arthritis (5)

The albumin concentration was decreased in only 2 subjects including one with symptoms of hepatic failure This patient also had the highest gamma globulin level These findings made us suspect chronic hepatitis or liver cirrhosis

The next highest gamma globulin levels were found in two women with purpura of the legs These cases were interpreted as having hyperglobulinaemic purpura

The Waler-Rose and the FIIA tests are positive in about 70 % and 80 % respectively in hospital patients with rheumatoid arthritis (5) In 72 cases with more or less clearcut systemic lupus, Leonhardt (6) found the results of both tests to be positive in 33 % Waldenstrom et al (16) found the Waler Rose test to be positive in about 20 % of a liver cirrhosis series and especially in the cases with a high gamma globulin concentration Rheumatoid factors were found in most of our cases

Since the subjects described were in relatively good health further investigations (liver function tests liver biopsy, L E cell test coagulation studies etc) were not considered well advised Our diagnoses were therefore based principally on the clinical history and the physical findings The conditions found were those expected

in a series of hypergammaglobulinaemia Chronic liver disease, rheumatoid arthritis, drug allergy, parotid swelling and hyperglobulinaemic purpura

Purpura hypergammaglobulinaemica first described by Waldenstrom (10, 11), is a relatively uncommon finding especially in its primary form Among 72 cases reported Struass (7) found 18 in which purpura was described as an isolated phenomenon The symptoms of hyperglobulinaemic purpura are probably often so mild that medical advice is not sought This makes it difficult to estimate the true frequency from hospital series In a random population we found two cases among seven thousand persons above 25 years Cases are however, described with a gamma globulin concentration below 20 g/100 ml (7) so that we may have missed some cases

Summary

Sera from 6995 persons who cooperated in a mass health control were studied by paper electrophoresis Pronounced (≥ 20 g/100 ml) polyclonal hypergammaglobulinaemia was found in 12 sera Nine of the 12 sera contained rheumatic factors and anti nuclear factors were found in two There was only one man in the group of persons from which these sera were taken As expected most of the subjects had a history of such conditions as rheumatoid arthritis liver

damage parolitis or drug allergy Two women had purpura hyperglobulin aemica

This study was supported by grants from Ernhold Lundström's Foundation, Malmö and Alfred Österlund's Foundation Malmö

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Monoclonal and Diclonal Gammopathies

By J W IMHOFF, R C BALLIEUX, N A J MUL and H POEN

— The immunoglobulins in normal human serum can be divided into four classes IgG, IgA, IgM and IgD. Every immunoglobulin molecule is made up of structural units known as L (for light) and H (for heavy) chains. The L chains are subunits which all four classes have in common. The H-chains, however, have a class specific structure and determine the immunochemical specificity of the class in question (1).

— Two structural types of L chains are known. In accordance with the corresponding types of Bence Jones protein, they can be designated types I and II (2). Since every immunoglobulin molecule contains two identical L chains, these proteins can be classified as type I or type II (3—6).

— Heterogeneity of the H chains has been established only for the IgG class (7—14) and is very likely, on the basis of Harboe's (15) work, to be the case also for the IgM class.

— In some diseases the serum can contain an abnormal globulin fraction often referred to as "paraprotein" or

M component. Such proteins have been mostly found in multiple myeloma and Waldenström's macroglobulinaemia, however, they can incidentally occur in many other diseases e.g. malignant tumours, reticulososes and collagen diseases (16—19) (table I).

— On the basis of the structural and immunological similarity to immunoglobulins or their structural units, the paraproteins¹ can be divided into classes and types.

— In view of MacFarlane Burnet's "clonal selection theory" Waldenström suggested the designation "monoclonal gammopathy" for paraproteinaemia (20). These cases are believed to be characterized by the existence of a single clone of immunologically competent cells such as lymphocytes or plasma cells, responsible for the production of one given type of protein (as manifested by the presence of a narrow banded hyperglobulinaemia or a paraprotein fraction in the elec-

¹ The term "paraprotein" is used in this study only as a synonym of "M component".

Table 1 Classification of M-components

Immunoglobulin classes	Immunological types in each class	
	I chain	H-chain
IgG	type I and II (γ or λ)	type γ_{1a} γ_{1b} γ_{1c} γ_{1d} or γ_{2c} γ_{2e} and γ_{2a} or type γ_e γ_e γ_1 and γ_e
IgA	type I and II	—
IgM	type I and II	—
IgD	type I and II	—
Bence Jones proteins	type I and II	—
Franklin proteins" (heavy chain or Franklin's disease)	—	type γ_r and γ_u

trophogram) One distinct abnormal protein fraction is found in the majority of patients with paraproteinaemia. It is attractive to refer to these cases as monoclonal gammopathies.

— In the course of the analysis of a large series of patients sera we observed besides the common form of single banded paraproteinaemia a number of patients with more than one serum paraprotein fraction. This paper intends to describe the combinations found and to present arguments in favour of the designation monoclonal paraproteinaemia with reference to some of these instances.

Material and methods

Sera from 120 patients with paraproteinaemia were examined. Agar electrophoresis was carried out according to Weme (21) and immunoelectrophoresis according to Scheffer (22).

For immunological typing of the paraprotein fractions antihuman serum of equine

Table II Classification and immunological typing of M-components in cases of single banded paraprotein aemia

	L Chain type		Not typed	Total
	I	II		
IgG	71	45	44	160
IgM	35	18	63	116
IgA	31	21	23	75
B J	12	9	5	26
Total	149	93	135	377

origin² specific anti IgG anti IgA and anti IgM serum, specific anti type I and type II L-chain serum all prepared in rabbits were used.

Results

— A single banded paraproteinaemia was established in 377 patients. The distribution over the various classes is shown in table II.

² Obtained from the Central Laboratory of the Bloodtransfusionservice of the Netherlands Red Cross — Amsterdam.

Monoclonal and Biclonal Gammopathies

By I W IMHOFF, R E BALLILYN, N A J MUL and H POEN

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¹ The term "paraprotein" is used in this study only as a synonym of "M component".

tains. The problem of the correlation between cellular structure and protein production has been approached by various techniques among which immunofluorescence microscopy and tissue cultures have made important contributions. On the basis of these studies it seems likely that under pathological conditions one given clone of proliferating immunocytes produces one distinct pathological protein. In these cases the "one clone/one protein theory" is acceptable as a working hypothesis at least if one protein is defined as one of the different types of immunoglobulins. But Osserman (16) holds that the one clone/one protein hypothesis is disputable until proven. On the basis of the demonstrated heterogeneity of the narrow banded protein components (24-26, 20) the possibility of a single clone/multiple proteins situation and even that of a multiple clones/multiple proteins situation remains open. However if at this moment we assume that the immunocyte dyscrasia reflects the neoplasia of an autonomic proliferation of a single cell clone resulting in the production of large amounts of a single class of immunoglobulins then we may refer to the single banded form of paraproteinaemia as monoclonal gammopathy (20).

— In some cases of paraproteinemia the patient's serum contains two or several paraprotein components of two different classes of immunoglobulins. In 1939 Curtin (27) described the co-occurrence of $I_{\kappa}M$

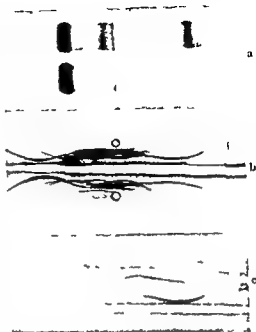


Figure 1 Analysis of the serum of case D. In each slide the patient's serum is on top and normal human serum as comparison on the bottom.

- a Agarose gel electrophoresis note the two M components
- b Immunoelectrophoresis the antiserum used was horse anti human serum. The paraproteins are both of the G type
- c Immunoelectrophoresis the antiserum in the upper trough is rabbit anti λ serum (anti type II L-chains). The antiserum in the lower trough is rabbit anti κ serum (anti type I L chain). The faster moving M component is of the immunological type II the slower moving one is of type I

and $I_{\kappa}G$ paraproteins in the same patient.

— We have previously reported on several patients in whom a dual paraproteinaemia was found (28-29). Bachmann and Laurell (30) also mentioned the occurrence of paraproteins

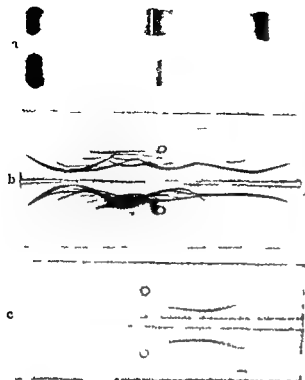


Figure 1 Analysis of the serum of case II In each slide the patient's serum is on top and normal human serum as comparison on the bottom

- a Agar gel electrophoresis note the two M components
- b Immuno electrophoresis the antiserum used was horse anti human serum The paraproteins are of the M type and of the G type respectively
- c Immuno electrophoresis the antiserum used was rabbit anti A serum (anti type I L chain)
Both M components are of the immunological type I The precipitation line of the IgM fraction is indicated by the dotted lines as the photographic reproduction is poor

— L chain typing was carried out (23) in 242 of these sera the results are presented in table II

— The electropherogram of 13 sera revealed more than one abnormal protein fraction Further analysis discloses

ed that co occurrence of a paraprotein fraction and a Bence Jones protein fraction of the same type existed in 7 cases Of these, 4 were of the IgG class (all being of the immunological type II), while the remaining 3 were of the IgA class (all abnormal protein fractions being of type I) In 5 cases, two paraprotein fractions of different class were detected in the same patient's serum The combinations found were

- A IgM^I+IgA^I
- B IgG^I+IgM^I
- C IgG^I+IgA^I (twice)
- D IgG^I+IgG^{II}s

In one case, a G myeloma protein of type I was detected in the serum whereas in the patient's serum and urine a type II Bence Jones protein could be demonstrated

- E IgG^I+Bence Jones^{II}

— The results of the immuno electrophoretic studies in cases B D and E are presented in figures 1 2 and 3

Discussion

— In the majority of cases of paraproteinemia the patient's serum contains a single abnormal protein fraction All these cases are characterized by immunocyte dyscrasia and it is probable if not certain that the proliferating immunocytes (lymphocytes plasma cells) produce the paraprotein

² We thank Dr J W Stoop Department of Pediatrics State University Utrecht for making available to us the serum of patient D

proteinemia whose urine contained Bence Jones proteins of type I as well as type II

— In one of our patients (E), we demonstrated the co occurrence in the serum of an IgG paraprotein component with a type I L chain and a Bence Jones protein of type II

— The investigations made by Bernier and Cebra (33) tend to indicate that the different types of L chain are produced by different cells Pernis and Chiappino (38) however reported on immunofluorescence experiments showing that in the white pulp of the spleen and in the germinal centres of the lymph nodes virtually all cells reacted with both types of L chain antisera This might indicate that the immunocytes in the germinal centres produce both L chain types At advancing maturation differentiation in protein production occurs the mature immunocytes producing only one type of L chain and one class of immunoglobulins [Bernier and Putnam (39)]

The findings in our patient and those described by Ingle and Nachman (37) might be explained by proliferation of two clones of mature immunocytes each clone producing a different type of L chain this would make the condition a clonal gammopathy An alternative possibility is that the paraproteins of different immunological types resulted from proliferation of a primitive clone of immunocytes still capable of producing both L chain types A very recent communication of Pernis (40) however makes this assumption unlikely

All things being equal the same applies to case D, in which the serum contained two paraprotein fractions both of the IgG class L chain typing disclosed that the different IgG paraproteins were made up of different L chains (IgG^I and IgG^{II})

— Studies are now being carried out to establish whether these cases involve a clonal paraproteinaemia or whether proliferation of a single clone of primitive immunocytes leads to the production of two different types of paraproteins

Summary

— A review is presented of 390 cases of paraproteinaemia including 377 which involved a single paraprotein fraction (monoclonal gammopathy) The M components were classified as IgG in 160 IgA in 75 IgM in 116 and Bence Jones in 26 instances

— In 13 cases the patient's serum contained two or several paraprotein fractions In 7 cases the serum contained in addition to a G or A myeloma protein a Bence Jones protein of the same immunological type In the remaining 6 cases the following combinations were found

$I_{\kappa}M^I + I_{\kappa}A^I$
 $I_{\kappa}G^I + I_{\kappa}M^I$
 $I_{\kappa}G^I + I_{\kappa}A^I$ (twice)
 $I_{\kappa}G^I + \text{Bence Jones}^{II}$
 $I_{\kappa}G^I + I_{\kappa}G^{II}$

— The possibility that a clonal gammopathy existed in these cases is discussed

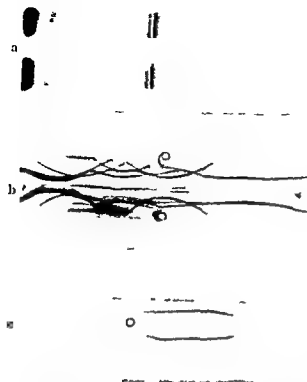


Figure 3 Analysis of the serum of case L. In each slide the patients serum is on top and normal human serum as comparison on the bottom

- a Agarose electrophoresis note the two M components
- b Immunoelectrophoresis the antiserum used was horse anti human serum A G paraprotein fraction is present with a slower moving Bence Jones protein
- c Immunoelectrophoresis the antisera used were the same as in 2 c The faster moving M component is of the immunological type I the slower moving one is of type II

of different classes in the same patient

Kirstner and Norberg (31) reported on a patient with two paraprotein fractions of the IgA and IgG class respectively

— In the light of immunofluorescence investigations carried out by Curtain et al (27), by van Furth (32)

and by Bernier and Cebra (33) it is highly probable that paraproteins of different classes (IgG, IgA and IgM) are produced by different cells. It seems justified, therefore, to use the designation *clonal gammaopathy* with reference to combinations of these paraproteinaemias. In the patients' sera which we examined and presented under headings A, B and C, a clonal paraproteinemia existed in our opinion. Evidence supporting this hypothesis is found in the fact that two different proliferating cell populations were found in the patients of groups A and B (i.e. plasma cells or myeloma cells and lymphatic cells in the bone marrow).

— A paraproteinemia is not infrequently associated with Bence Jones proteinuria, while the serum contains a Bence Jones protein fraction besides the paraprotein component. It can be assumed that in these cases the proliferating immunocytes produce an excess of free light chains. In these cases which combine an abnormal serum protein component (IgG, IgA or IgM) with Bence Jones proteinuria or proteinuria, the L chains of the serum component are nearly always of the same antigenic type (I or II) as the corresponding Bence Jones protein (34–36).

— In all these cases it is plausible that a single clone of neoplastically proliferating immunocytes produces an excess of L chains. We found such a combination in 7 patients.

— Engle and Nachmann (37) reported on a patient with IgG¹ para-

proteinæmia, whose urine contained Bence Jones proteins of type I as well as type II

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IgM^I + IgA^I
IgG^I + IgM^I
IgG^I + IgA^I (twice)
IgG^I + Bence Jones^{II}
IgG^I + IgG^{II}

— The possibility that a clonal gammopathy existed in these cases is discussed

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The Structure of Waldenström Macroglobulins¹

By IRANK W. PUTNAM, NITSLO KOZLURU and CAROLINE W. CASLEY

Although the high molecular weight 19S globulins discovered in the serum of patients with macroglobulinemia by Waldenström (10) are among the largest of the well characterized proteins study of their primary structure is possible because they are polymers of subunits containing two kinds of polypeptide chains — heavy and light. Since the light chains are structurally related to Bence Jones proteins whose amino acid sequence is known, determination of the tryptic peptide maps permits deductions as to the actual sequence of this portion of the light chains which is shared with Bence Jones proteins. This study is facilitated by the relative homogeneity of individual Waldenström macroglobulins and the large amounts obtainable because of the hyperglobulinemia and the frequent use of plasmapheresis as a therapeutic procedure. However a unique solution to the structural pro-

blem is precluded by the existence of two antigenic types (I and II) and the finding that macroglobulins from individual patients differ in structure. The significance of structural study of Waldenström macroglobulins lies in the contribution to be made to the understanding of the variability of γ globulins and the biosynthesis of antibodies.

For detailed structural study one must obtain well defined reproducible bits of the original molecules. In the case of Waldenström macroglobulins reductive dissociation of the polymers yields monomer units with a molecular weight of about 160 000 — much too large for direct study. Two further cleavage procedures have provided the necessary bits: 1) partial enzymatic cleavage with papain and 2) separation of the light and heavy chains by reduction and alkylation. In each case the cleavage products are digested with trypsin and the tryptic peptide maps prepared by standard procedures (7).

Papain cleavage of Waldenström macroglobulins does not yield two

¹ Supported by research grants (CA 02603 and CA 0812) of the National Cancer Institute National Institutes of Health Bethesda Maryland.

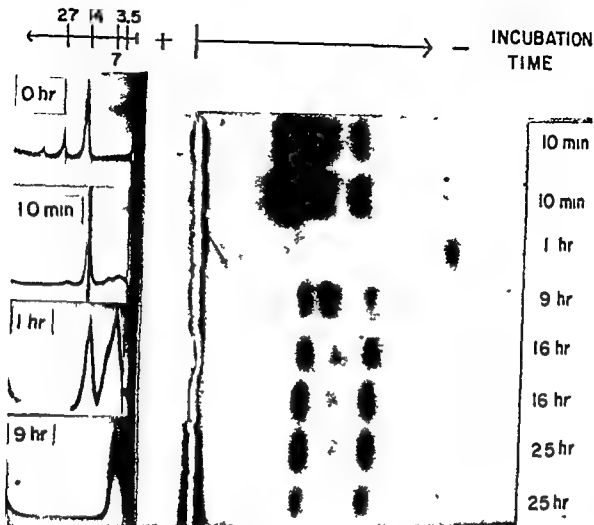


Fig 1 Composite illustration of the change in ultracentrifugal pattern and the starch gel electrophoretic pattern of a macroglobulin incubated with papain for increasing periods of time *Left* Ultracentrifugal pattern of the original macroglobulin (0 hr) and of the digests at 10 min 1 hr and 9 hr The direction of sedimentation and the approximate s_{20} are given at the top of the photographs The photographs are at different bar angles but were all taken at the same time after full speed was reached (16 min at 50780 rpm) *Right* Starch gel electrophoresis at pH 5.0 at 250 volts for 6 hr of aliquots of the digest incubated for increasing times The direction of migration from the sample slot is given by the arrow In this experiment no protein moved towards the cathode and any intact macroglobulin remained in the starting slot

well defined parts of the molecule with different chemical and biological properties as in the case of 7S γ globulins However through study of the papain digest of one macroglobulin our laboratory first recognized the an-

tigenic classification of myeloma globulins and macroglobulins into antigenic types I and II corresponding to the two antigenic types of Bence Jones proteins (6) Figure 1 illustrates the change in ultracentrifugal pattern and



Fig. 2. Tracing of the peptide pattern of the tryptic digest of an oxidized macroglobulin of antigenic type I (Na) as developed with ninhydrin and with the specific staining reagents identified in the figure legend. Chromatography is in the vertical direction, while electrophoresis at pH 3.7 is in the horizontal direction. Peptides designated B₁, etc. are present in most Bence Jones proteins of antigenic type I in normal human γ globulin and in myeloma globulins of antigenic type I. Weak spots with ninhydrin are indicated by dotted lines.

starch gel electrophoretic pattern of an antigenic type I macroglobulin (specimen Na) incubated with papain for increasing periods of time. The polymer peaks disappear with the rapid formation of a 3.5 S component which represents all the ultracentrifugally detectable protein at the end of 1 hour. Since the high molecular weight polymers are excluded from the starch gel the papain cleavage products are demonstrable at ten minutes. Finally, three electrophoretic bands remain. By the Ouchterlony method and by starch gel immunoelectrophoresis these appeared antigenically identical. By chromatography it was demonstrated that the whole papain digest consisted of low

molecular weight dialyzable peptides, a higher molecular weight glycopeptide and a 3.5 S fraction that gave the three banded starch gel electrophoretic pattern illustrated in Fig. 1. From antigenic studies and from the peptide map illustrated later we concluded that sulfhydryl activated papain dissociates the polymer into subunits, degrades a part of the heavy chain to low molecular weight peptides and leaves a series of closely related 3.5 S fragments that represent the light chain plus part of the heavy chain.

Historically the most significant result obtained from papain digestion of macroglobulins was the recognition of the antigenic classification of all human immunoglobulins into two antigenic types. Antiserum prepared in rabbits against the papain digest of the Na macroglobulin unexpectedly proved to be the best reagent for antigenic typing of Bence Jones proteins. The antiserum reacted strongly with all type I Bence Jones proteins but not at all with type II. This suggested to us that Waldenström macroglobulins were either antigenic type I or II. Further study revealed that myeloma globulins likewise could be divided into antigenic type I or II (6). This led to the classification of all pathological human serum γ globulins into the same two antigenic types as Bence Jones proteins, a conclusion independently arrived at in several laboratories (3, 7).

The antigenic similarity of the papain fragment of the antigenic type I macroglobulin Na to type I Bence Jones proteins prompted a study of

their structural relationship by the "fingerprint" method. Figure 2 illustrates the tryptic peptide map of the performic acid oxidized macroglobulin Na. Whereas there are about 120 moles of arginine and lysine per monomer of 160,000 molecular weight, only about half as many spots are discernible in the figure. This suggests the presence of several subunits within the monomer, namely, the heavy and light chains.

By the use of multiple staining procedures (2) and mixtures of the tryptic digests of this macroglobulin and various type I Bence Jones proteins, fifteen peptides could be identified as present in the latter and thus representing the light chains of the macroglobulin. These peptides are identified in Fig. 2 by the designations B₁, B₂, etc. Of the peptides so identified in Fig. 2, the complete amino acid sequence has been reported for the following peptides that have been isolated from a type I Bence Jones protein: B₁, B₃, B₆, B₇, B₈, B₉, B₁₀, B₁₁, B₁₃, B₁₄, B₁₅, B₁₆, and B₁₈ (7, 9).

B₃ is the amino terminal octadecipeptide of many type I light chains; it begins with aspartic acid, has the only methionine residue in the light chain, and its sequence is known (8). B₃ and B₁₆ are in the variable portion of the light chain just preceding the COOH terminal half, the latter which seems to be almost invariant in type I light chains; contains the peptides B₁₈, B₁₁, B₁₀, B₆, B₁₅, B₇, B₉, and B₁ (9). Thus the amino acid sequence of the light chain of the macroglobulin Na whose peptide map is illustrated

in Fig. 2, is very similar to that of a type I Bence Jones protein for which the amino acid sequence is almost completely known. In general, the light chains of type I microglobulins are very similar in primary structure to type I Bence-Jones proteins. However, since the latter differ individually in sequence in their amino terminal halves, but are almost identical in their carboxyl terminal halves (9), it was necessary to compare the peptide maps of the light chains of a number of type I macroglobulins. To do this separation into the light and heavy chains was required.

The light and heavy chains of four type I macroglobulins and one type II macroglobulin were dissociated by reduction and alkylation by the method of Fleischman *et al.* (4) followed by separation on a G 100 Sephadex column. The purity of the fractionated chains was established by starch gel electrophoresis, and by immunodiffusion with antiserum specific for the heavy chains of γ G and γ A globulins and for type I and II light chains, as well as the intact globulins of the three major antigenic classes (γ G, γ A, and γ M). On the basis of optical absorbancy at 280m μ , the light chains constituted about 25% of the macroglobulin molecule and the heavy chains about 75%. From amino acid analysis of four microglobulins of type I it was evident that the heavy and light chains differ markedly in composition but are complementary with respect to the whole macroglobulins. In every case where the heavy and light chains differed significantly



Fig 3 Peptide maps of a type I macroglobulin (Di) in the center and of the heavy chain (left) and the light chain (right). The peptides were developed with ninhydrin and the circled peptides were positive for arginine. In the central map the arrows indicate peptides due to the light chain in the map for the light chain the peptides denoted B₁ etc., correspond to similarly designated peptides in Fig. 2. The conditions are as in Fig. 2 except that the macroglobulin and its polypeptide chains were reduced and alkylated. This changes the position of sulfur containing peptides such as B₁ and tryptophan containing peptides such as B₁₀.

in amino acid composition the analytical values for the whole macroglobulin were intermediate.

The complementarity of the heavy and light chains is illustrated by the peptide maps of Fig. 3. The center photograph is the peptide map of the soluble tryptic peptides of the 19S macroglobulin from a patient (Di) with Waldenström macroglobulinemia. The left and right panels represent respectively the heavy (H) and light (L) chains. It should be noted that the map of the intact macroglobulin is almost a faithful composite of the maps of its constituent heavy and light chains. For example, the glycopeptides (the two dark spots on the abscissa near the origin) are present only in the heavy chain and the whole macroglobulin where is certain peptides that are found in the map of the whole macroglobulin become stronger in the maps for the individual

chains. One example is B₄, a peptide containing 20 amino acids for which the order has been determined (positions 148—167 in the sequence given by Tatum et al. (9)). In principle the sequence of type I macroglobulins will be about one third determined when the sequence of type I Bence Jones proteins is completed.

Comparative study of the peptide maps of the light chains of four type I macroglobulins confirmed their structural relationship to Bence Jones proteins as evidenced by the sharing of the H peptides. The comparative study likewise supported the concept that the light chains of macroglobulins from individual patients differ from each other in a number of peptides though possessing many peptides in common. As in the Bence Jones proteins structural differences seemed to be localized in the amino terminal portions of the chains. For

example, in two macroglobulin light chains the amino terminal peptide B₃ was present, this begins with aspartic acid and contains methionine (8). However, the light chains from two other macroglobulins both lacked the amino terminal peptide B₃. One chain began with aspartic acid but lacked methionine, the other light chain began with glutamic acid and contained methionine. Thus, there are at least three ways in which type I macroglobulin light chains can begin. However, all light chains and Bence-Jones proteins of type I appear to end in the same way because they all contain the carboxyl terminal peptide B₁ (Gly Glu CysH). The type II light chains differ greatly in structure from the type I light chains but seem to be made up on a similar principle.

Although the structural study of macroglobulin light chains is well advanced, only comparative study of the heavy chains by the peptide map method has been undertaken. As in the case of γ A heavy chains (1) the heavy chains of Waldenström macroglobulins share many peptides but differ in many others. It appears as if a portion of the heavy chain is shared by all macroglobulins and another portion

differs for the individual patient. Future studies are needed to define the role of the heavy and light chains, to establish the location and function of the interchain and intermolecular disulfide bonds, and to elucidate the structure of the carbohydrate moiety. Ultimately, the structural study of Waldenström macroglobulins should contribute greatly to knowledge of the structure and biosynthesis of antibodies.

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Studies on the Macroglobulins of Human Serum II Heterogeneity of Antigenic Determinants Among M- Components in Waldenström's Macroglobulinemia¹

By FRANK A. WOLLHEIM and RALPH C. WILLIAMS JR.

Starting with the observation by Oudin (1a) on isotyping among rabbit γ immunoglobulins an increasing number of serum proteins have been shown to exhibit genetically determined subgroups. The Gm groups (9) are well known in this regard. Recently subgroups of human γ chains (3) have been described independently in the laboratories of Kunkel (7) Fahey (18) and Putnam (1) and these have shown correlation to the previously mentioned Gm groups (12). The possibility of subgroups of the other classes of immunoglobulins is attracting increasing attention. IgA is known to show allo types in mice (10). Seligmann (16) has reported familial occurrence of macro M components and further more obtained antisera that would react with some but not with other such M components. Some insight into the heterogeneity of macroglobulin antigens was presented by Deutsch

and Mackenzie (4). By immunizing monkeys with IgM Rh antibodies from individual sera anti IgM antisera were produced which reacted with a varying number of a panel of other 19 S Rh antibodies. Similarly our own experiments utilizing heterophile sera from patients with infectious mononucleosis showed marked variation in patterns of inhibition by different anti IgM antisera prepared in rabbits (20). The present investigation was undertaken in an attempt to study the antigenic structure of 8 monoclonal IgM proteins and the heavy and light chains prepared from them. Utilizing rabbit antisera to individual macroglobulin M components evidence was obtained not only for the well known homologous individual specificity (8) but for varying degrees of cross reactivity with heterologous chains thus indicating the existence of subgroups among both heavy and light chains. In addition it was a constant finding that the native IgM molecule always contained antigenic determinants in ex

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cess of both isolated chains and mixtures of heavy and light chains, in other words, the native whole structure or undissociated macroglobulin contained antigens not found in its parts or chains produced by reduction and alkylation.

Materials and Methods

All eight sera utilized in this study showed a well defined monoclonal spike on paper and agar electrophoresis. The M component was isolated and shown to be immunologically pure IgM by immunoelectrophoresis. In two instances (Ka and Ro) the whole sera gave good euglobulin precipitates on dilution with 9 parts of water and this was used as the first purification step. The other six M components were isolated by starch block electrophoresis (11). Final purification was achieved by chromatography on DEAE Sephadex G50 using stepwise elution with 0.15 M PO_4 pH 6.8 and 0.3 M PO_4 pH 6.8. The pure IgM occurred in the last step. Rabbit antisera were prepared by immunizing with isolated Waldenström's IgM proteins in complete Freund's adjuvant followed in some instances by intravenous injections of alum precipitated antigen. Macroglobulin μ and I chains were prepared in the following way adapted after Fleischman, Pain and Porter (5): 10 mg of the isolated macroglobulin in 2–4 cc 0.15 M NaCl were mixed with an equal volume of 0.2 M Tris buffer pH 8. 0.2 M PDTA was added to give a final concentration of 0.032 M. The mixture was degassed and put under an atmosphere of nitrogen. 2 Mercaptoethanol was then added to a concentration of 0.1 M and the mixture left at room temperature for 60–90 minutes. It was then cooled in ice water and a slight molar excess of iodoacetamide was added. After another 60–90 minutes at 0° C dialysis vs 0.5 M propionic acid was started. This was continued for 16–20 hours with one change of acid after 3 hours. The reduction mixture was then concentrated by negative pressure dialysis to 1 cc and

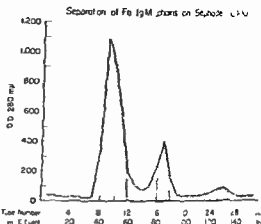


Figure 1 Typical gel filtration chromatogram obtained after reduction alkylation and dialysis of 10 mg pure macro M component. A 12×140 cm column with Sephadex G100 was equilibrated and eluted with 0.5 N propionic acid. Peak 1 (6–7 mg protein) contained heavy chains with no or only trace reactions with anti light chain antisera. Peak 2 contained light chains. Peak 3 contained no identifiable antigenic determinants.

applied to a Sephadex G100 column measuring 12×140 cm equilibrated with propionic acid. Figure 1 shows a typical elution pattern. The peaks were pooled, dialyzed against 0.1 M acetate buffer pH 5.5, concentrated to 1–3 mg protein per cc and stored at –20° C. Ouchterlony double diffusion experiments were performed in 2% agar at 4° C and the plates were observed for at least 1 week. Tanned sheep cells coated with IgM heavy chains were prepared after the method of Boyden (2). Inhibition experiments were performed by incubating 0.1 cc aliquots of various rabbit anti IgM antisera with 0.1 cc IgM preparations in serial dilutions for 30 minutes at room temperature before adding 1 drop of the 1% tanned cell suspension. The tubes were read after overnight standing at 4° C.

Results

Figure 2 shows a typical pattern obtained by double diffusion of λ IgM and chain preparations of this protein.

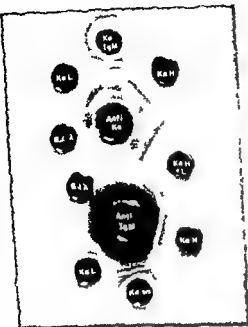


Figure 2 Ouchterlony experiment on ka. Anti ka rabbit anti ka IgM absorbed with cord serum. Anti IgM=rabbit antiserum to polyclonal IgM absorbed with cord serum. Ka W=ka whole serum. Ka IgM=isolated M component. Ka H=ka heavy chains. Ka L=ka light chains. Ka H+L=mixture of H and L. Bence Jones λ =isolated Bence Jones protein of group λ . The ka IgM was typed as λ .

with in homologous and one heterologous antiserum both absorbed with human cord serum. It can be seen that the intact IgM spurs over both its heavy and light chains and this was also true in relation to unseparated mixture of alkylated chains. It can also be seen that the homologous antiserum reacts with the L chains despite the fact that kappa and lambda determinants were absorbed out with cord serum. Similar patterns were obtained with unalkylated IgM prepara-

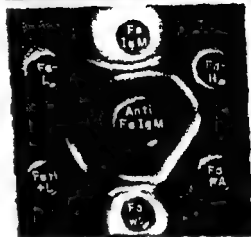


Figure 3 Ouchterlony experiment on Fa. Anti Fa IgM=anti Fa macro M component absorbed with cord serum. Fa W=Fa whole serum. Fa IgM=isolated M component. Fa H=heavy chains. Fa L=light chains. Fa H+L=chains mixed after initial separation.

tions and a variety of anti IgM antisera. Figure 3 shows the reactions between anti Fa and Fa IgM and Fa chains. It can be seen that the unreduced IgM already contains some heavy chain like material. The same situation was also observed with ka IgM.

Having thus established the fact that whole IgM has antigenic determinants



Figure 4 Ouchterlony experiment with 8 different IgM heavy chain preparations A = anti Ro IgM absorbed with cord serum B = anti Ro IgM absorbed with cord serum and kei whole serum

n units lost during reduction, attempts were made to detect antigenic subgroups. In general specific anti IgM antiserum prepared against monoclonal Wildenstrom's macroglobulins and absorbed with cord serum reacted with all 8 purified IgM preparations in addition to showing individual specificity for the homologous IgM proteins. These results are similar to those obtained by Grey, et al (8) with M components of the IgG, IgA and IgM subclasses of immunoglobulins. Absorption of such antiserum with small amounts of heterologous whole macroglobulinemia serum resulted in loss of the precipitation reaction with all 8 intact IgM preparations. However such absorbed antiserum were still able to react with heavy chains from some but not from other IgM globulins. Figure 4 shows the result of a typical experiment. Table I summarizes the results obtained thus far with the 8 heavy chains of the panel. Where is

Table I Precipitin Reactions of IgM heavy chains (μ) in Ouchterlony double diffusion with anti IgM Ro absorbed with cord serum, or with cord serum and macroglobulinemia serum, and with some other cord serum absorbed anti monoclonal IgM antiserum

Antiserum	IgM heavy chains 10 mg/cc							
	Li	Ma	Kei	Ro	Li	Ma	Kei	Ro
Anti Ro abs cord	+	+	+	+	+	+	+	+
Anti Ro abs L ₁ H	—	—	—	+	+	+	+	+
Anti Ro abs L ₁	—	—	—	+	—	+	+	+
Anti Ro abs Kei	—	—	—	+	—	+	+	+
Anti Ro abs Ma	—	—	—	—	—	—	—	+
Anti Ro abs Li	—	—	—	—	—	—	—	+
Anti Ro abs Ma	—	—	—	—	—	—	—	+
Anti Ro abs No	—	—	—	—	—	—	—	+
Anti Ma abs cord	—	—	+	+	—	+	+	+
Anti Li abs cord	+	—	+	+	—	+	+	+
Anti Kei abs cord	+	—	+	+	+	+	+	+

anti Ro absorbed with cord serum only reacted with all 8 μ chains of the panel the similarly absorbed anti Ma failed to precipitate with Li, L₁H and Kei μ chains the anti Li reacted with all except L₁H and Kei and the anti Kei with all except L₁H. Absorption of anti Ro with either L₁ or Kei whole serum resulted in identical antiserum reacting with only Li, Ma, No and Ro. Absorption with Li, Li, Ma and No all gave antisera only reacting with the homologous Ro μ chains thus leaving only antibodies to the individual μ chain determinant of Ro. It is evident from these experiments that Li, Ma, No and Ro form a closely related subgroup. The existence of other subgroups is suggested by the reactions with anti Li and anti Kei

Table II Inhibition of sheep erythrocytes, tanned with L'H heavy chains
Agglutinator 1:500 dilution of anti her IgM Agglutinator and inhibitor in
cubated for 30 mins before addition of test cells¹

Inhibitor	Concentration mg/ce													
	1	2	12	003	032	016	008	004	002	001	0005	00025	00013	00006
Fe IgM	NT	—	—	—	—	—	—	—	1	1	1-2		2	3
L'H IgM	—	—	—	—	1	1	1-2	2	2	3	3	3	3	3
Ii IgM	—	—	—	—	1	2	2	3	3	3	3	3	3	
her IgM	—	—	—	—	—	—	—	—	1	1	1-2	3	3	3
Ma IgM	—	—	—	—	—	1	2	2	3	3	3	3	3	3

¹ Reactions were read after overnight incubation in the cold room in a one to three scale

but our data with these antisera are
is yet incomplete

The light chains reacted in general
only with their homologous antise-
rum but an exception from this rule
was anti Ro which reacted with light
chains from her Ma and weakly with
Ii and Li (Figure 5)

Table II shows the result of an
hemagglutination inhibition experi-
ment with L'H heavy chains tanned
on sheep cells anti Ii and whole IgM
preparations as inhibitors. It can be
seen that Ii and her were most potent
inhibitors. Ma was intermediate and
L'H inhibited most weakly. It
was thus possible to show serological
differences among M components
which could be confirmed in agar dif-
fusion experiments with the same in-
titerum. Technical difficulties have
thus far not permitted us to expand
experiments of this type.

Discussion and conclusions

In the present study reduction and
alkylation of human IgM globulins
gave rise to heavy and light chains in
about the same ratio as in the case

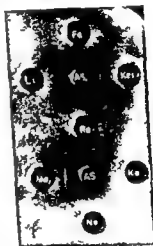


Figure 5 Ouchterlony experiment with 7
different IgM light chain preparations AS =
anti Ro IgM absorbed with cord serum.

with IgG. This may indicate a similar
structure of these two proteins. How-
ever Ouchterlony antigenic analysis
has shown that major precipitating
antigenic determinants are lost upon
such reduction implying that intact
disulfide bonds (between heavy and
light chains) or relatively labile intra-
chain disulfides are essential for sta-
bilizing or shaping much of the speci-
fic IgM antigenic site. The conforma-

tion of the native IgM molecule thus may be of paramount importance for antigenicity, much in the same fashion as has been shown to be the case with ribonuclease (14). It has been shown, that reduction of three disulfide bonds in the single chain of this polypeptide without any loss of amino acids completely destroys the reaction with specific antisera. Similar observations have recently been made by Gerstein, et al (6) with respect to pepsinogen.

The fact that all individual IgM preparations in our experience reacted with all strong antisera to intact monoclonal IgM, as well as that absorption with one IgM abolished reactivity with all other intact IgM preparations indicates a high degree of cross reactivity among these M components. On the other hand the heavy chains derived from them show marked differences in antigenic specificity, and seem to fall into subgroups that are hidden in the intact IgM molecule. This H chain subgrouping appeared to be a relatively buried antigenic determinant since it was brought out by anti-IgM antisera absorbed with whole macroglobulin sera.

The sensitive serological method of hemagglutination inhibition is probably more suited to detect differences of the antigenic structure of whole IgM, as indicated in Table II. This view is also supported by observations of Deutsch and MacKenzie (4) on monkey antisera to IgM Rh antibodies and by the present authors in inhibition of IgM heterophile antibodies with antiserum to monoclonal IgM (20).

MacKenzie and Deutsch (13) have in their recent paper further extended their observations on inhibition of monkey antisera to individual anti-IgM Rh antibodies and Waldenström's macroglobulins. They found the same phenomenon of differences in inhibition of individual antibodies from different sera to be true also of anti-A and anti-B mercaptoethanol sensitive blood group antibodies, but not on the whole of cold agglutinins. Whereas the monkey antisera used by these authors thus behaved very similarly to our rabbit antisera in inhibition of serologically active IgM antibodies they failed apparently to recognize both the hidden individual light chain determinants (19) and the H chain determinants described in this paper. This failure to precipitate chains could be due to either the technique used for immunization or to the animal species used. It is also surprising to note absence of individual specificity of their anti-Waldenström antisera for the homologous whole IgM.

It is not surprising that the antigenic heterogeneity of light chains now known to involve subgroups of the original K type (17) also seems to be present among light chains derived from IgM. Our data in this regard are in accordance with Solomon's et al observation as well as those obtained by tanned cell inhibition (19) in previous work from this laboratory.

Summary

Human monoclonal IgM was reduced in 0.1 M 2-mercaptoethanol and heavy

and light chains were obtained by gel filtration. It was found that a large part of reactivity with anti IgM antisera was lost on reduction and alkylation alone. On the other hand anti IgM antisera could be absorbed with whole sera free of anti IgM but still retain reactivity with some heavy chains from individual M components. Evidence was thereby obtained for an isomeric heterogeneity among heavy chains indicating several subgroups. Further evidence was obtained for the heterogeneity of the intact IgM molecules from tanned cell inhibition experiments. Subgroups of the light chains within the kappa group were also present.

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and light chains were obtained by gel filtration. It was found that a large part of reactivity with anti IgM antiserum was lost on reduction and alkylation alone. On the other hand anti IgM antisera could be absorbed with whole sera free of anti IgM but still retain reactivity with some heavy chains from individual M components. Evidence was thereby obtained for antigenic heterogeneity among heavy chains indicating several subgroups. Further evidence was obtained for heterogeneity of the intact IgM molecules from tanned cell inhibition experiments. Subgroups of the light chains within the kappa group were also present.

Acknowledgements

The authors are indebted to Dr R Green, Dr H Poleski and Dr H Zinnemann for making IgM sera available. The technical assistance of Mrs Janice Larsen is gratefully acknowledged.

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A New Immunological Test for Monoclonal Macroglobulinemia — A Preliminary Report

By LEONHARD KORNIGOLD

In 1944 Waldenström (3) described the first cases of macroglobulinemia — a syndrome characterized by a tremendous increase in a γ globulin with a sedimentation constant of 19 S. This protein, which has a molecular weight of 1,000,000 is now known to be a member of the immunoglobulins and is designated as IgM or γ M. In 1962 Waldenström suggested that conditions giving rise to unusual concentrations of the immunoglobulins be called gammopathies (4) and that they be subdivided into polyclonal or monoclonal gammopathies according to their electrophoretic pattern — i.e. whether the immunoglobulin region was diffuse (polyclonal) or characterized by a narrow spike (monoclonal).

During the last few years it has been established that all immunoglobulins can be subdivided into two antigenic

types, now designated H and L, and that these antigenic configurations reside in the light chains, which are common to all immunoglobulins (1).

Our laboratory has been interested in devising immunologic tests for the gammopathies based on the fact that in the polyclonal gammopathies both antigenic types become proportionally elevated whereas, in the monoclonal gammopathies one of these types becomes predominant (2).

Moreover, we wanted to do this with antisera that could distinguish between the γ G, γ A and γ M gammopathies without having to isolate the suspected immunoglobulins. This has been accomplished without much difficulty for the γ G and γ A gammopathies. The γ M gammopathies on the other hand presented problems, which seemed unsurmountable for theoretical reasons. Since the detection of the H and L types of the γ M proteins depended on the presence of antibody against either the kappa or lambda chains the γ M globulins always had to be isolated in re-

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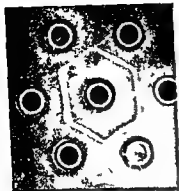


Figure 1 Ouchterlony gel diffusion plate Center anti γ M serum absorbed with γ G globulin Top left and bottom right normal serum (1 2) The other reservoirs contain sera (1 10) from patients with monoclonal gamma M pathologies of type k

latively pure form otherwise the smaller (and more rapidly diffusing) γ G proteins would interfere with the typing

The present paper will discuss the preparation of anti γ M antisera that lack antibodies against the light chains but which nevertheless can be used for typing the γ M globulins

Materials and Methods

γ M globulins — γ M globulins were isolated from the sera of patients with macroglobulinemia of Waldenström. Some of these proteins were cryoglobulins and were purified by repeated precipitation in the cold and solubilization at 3 °C. The other γ M preparations were obtained by preparative starch block electrophoresis.

Immunization — Rabbits were inoculated with the purified γ M proteins in complete Freund adjuvant until the antisera had high titers of antibody against γ M globulin.

Absorption — The anti γ M antisera were absorbed with γ G globulin in concentrations

of 2–10 mg γ G per ml of antiserum. When ever necessary these absorbed antisera were also absorbed with sera from agammaglobulinemic patients.

In addition some of these antisera were subsequently absorbed with a monoclonal γ M protein of the type different from that used for immunization.

Tests — All antisera were tested by the Ouchterlony gel diffusion technique. Purified γ M proteins as well as whole sera were employed as antigens.

Micro immunoelectrophoresis was performed as described elsewhere (2) either with agar or agarose gels.

Results

Relatively few of the antisera produced could distinguish between the two types of γ M proteins after absorption with γ G. Figure 1 illustrates the reaction of one such antiserum with normal serum, γ M and four sera from patients with macroglobulinemia of Waldenström. The antiserum was against a type L γ M protein and produced spurs whenever normal γ M or a mixture of γ Mk and γ ML was placed adjacent to a γ Mk containing serum. This spur formation is of course due to anti L antibody that cannot combine with k molecules.

Similar results were obtained with anti γ Mk sera, normal γ M and patients' sera of type γ ML. To date we have checked a large number of γ M proteins of known type with these antisera and in each instance they reacted according to expectation — i.e., proteins that reacted with antisera against kappa chains were always typed as K when checked with antisera against γ Mk and γ ML (absorbed with γ G) and as yet we have not encountered any

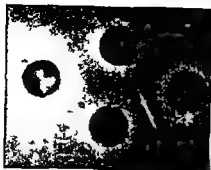


Figure 2 Ouchterlony gel diffusion plate
Top serum from patient with monoclonal
macroglobulinemia of type L (1:10) Bottom
serum from patient with monoclonal macro-
globulinemia of type k (1:10) Right specific
anti γ Mh left specific anti γ ML

discrepancies between the two typing methods

Absorption of these anti γ Mh or anti γ ML antisera with γ G and a monoclonal γ M of opposite type resulted in specific antisera. If an anti γ Mh anti serum absorbed with a γ ML protein would react only with γ Mh molecules. This is illustrated in figures 2 and 3. Note that the normal serum in figure 3 reacts with antisera against both types since it contains both γ Mh and γ ML. Moreover the lack of any reactivity with γ G or γ A is apparent.

To date all sera with antigenic monoclonal γ M were obtained from patients with malignancies usually lymphomas. We have encountered one serum from a patient with rheumatoid arthritis containing such large amounts of γ M that it was indistinguishable from sera of patients with macroglobulinemia of Waldenström by ultracentrifugal analysis and paper electrophoresis. Antigenically however, it was polyclonal i.e. both k and L γ M types were elevated.

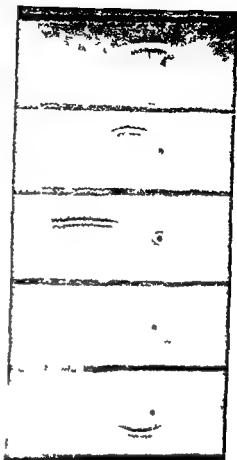


Figure 3 Immunoelectrophoresis on agarose gel. Anode is on left. The first three and the last hole contain sera from patients with monoclonal macroglobulinemia. The fourth hole contains normal serum. Its γ M globulin reacts with both specific anti γ M sera. The trenches contain alternately specific anti γ ML and anti γ Mh antisera. Consequently the first serum contains a γ ML globulin. Note that occasionally the patient's sera contain besides the monoclonal γ M also some normal γ M globulin of the other type.

Discussion

Two forms of hyper γ globulinemia — the electrophoretically diffuse and the electrophoretically homogeneous (spike) form have been known for a long time. These are now usually designated as polyclonal and monoclonal. In the former the elevation of

immunoglobulins is presumed to be caused by the proliferation of most of the immunoglobulin forming cells in the latter, the increase in immunoglobulins is the product of the offspring of one such cell which has become the predominant cellular population. The polyclonal gammopathies are usually a response to infectious or inflammatory diseases; the monoclonal gammopathies to malignant transformation. Consequently from a diagnostic point of view it is important to establish whether a hyper γ globulinemia is of the polyclonal or monoclonal variety. In the γ G gammopathies this may be accomplished by electrophoretic or immunoelectrophoretic methods. The γ M gammopathies are not as easily recognized since many monoclonal γ M gammopathies contain only slight increases of γ M globulin with frequent large increases of polyclonal γ G globulins. Consequently an immunological method capable of distinguishing between the different γ M gammopathies will be of great diagnostic value. More over studies on the composition of the immunoglobulins in the hyper γ globulinemias may shed light as to when (and why) a polyclonal gammopathy changes into a monoclonal gammopathy. The preliminary data presented here show that it is possible to distinguish between the two antigenic types κ and λ of the γ M globulins without any interference of the γ G and γ A globulins in the serum. Our data are also of theoretical interest since the antisera used in this work cannot react with the light chains which usually determine the κ or λ specificity of an

immunoglobulin. This finding suggests that either the heavy (μ) chain of the γ M protein contains a configuration characteristic of the κ or λ type or that the junction of the light chain to the μ chain results in a configuration that is determined both by the type of the light chain and the class of the heavy chain. Preliminary data favor the latter explanation i.e. the junction of a kappa chain to a μ chain forms a configuration detectable by antibody against a γ M κ globulin that can no longer react either with the isolated heavy chain or light chains.

Summary

Antisera against γ M globulins of either type κ or λ were absorbed with γ G globulins until they no longer reacted with γ G or the light chains of the immunoglobulins. Some of these antisera still could distinguish γ M globulins of type κ from those of type λ . These antisera are now being used to type sera from patients with suspected γ M gammopathies to determine whether they are antigenically monoclonal or polyclonal. Preliminary data suggest that those antibodies capable of distinguishing between the two types of γ M globulin react with a specific site produced by the junction of the light chains with the heavy (μ) chain of the macroglobulin.

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The author wishes to acknowledge the technical assistance of Mr Raul Vidal, Miss Heidemarie E. Berker and Mrs. Karen R. Chernis.

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A Thin-Layer Gel Filtration Technique for Proteins A Simple Clinical Method for Measuring Serum Macroglobulins

By KURT BERGSTROM¹

Gel filtration has become an established technique for separating proteins according to molecular size (1-5, 9). For analytical purposes thin layer chromatography techniques have been successfully applied especially since a loosely cross linked gel¹ (Sephadex G 200 Superfine) with appropriate particle size has become available (6, 7, 8).

The most accurate way of confirming the diagnosis of macroglobulinemia is to measure the amount of macroglobulins by ultracentrifugation. However gel filtration provides a less expensive and easier way of studying the protein molecules. As with ultracentrifugation serum can be separated into three main fractions according to molecular weight (or volume): 4-5% or the albumin group, 7% or the gamma globulin group and 19-25% or the macroglobulin group.

This paper is concerned with a thin

layer gel filtration technique for quantitative study of these three groups of serum proteins. It is recommended for quantitative studies of pathological sera as a simple and inexpensive complement to paper and immunoelectrophoresis and also for estimating the molecular size of purified proteins.

Materials and equipment

Gel Sephadex G 200 Superfine (lots To 23,4 and To 23,6) both with particle size 10-40 μ l was obtained from Pharmacia AB Lppsala Sweden.

Glass Plates Grooved commercial window glass available at any glaziers was cut the long way into 10x23 cm rectangles (cross section shown in Figure 1). Each plate had seven grooves 23 cm long which served the same purpose as stripping the supporting media in other chromatographic techniques.

Reference Substances Blue Dextran 2000 — average molecular weight 2 000 000 (Pharmacia AB) is used in solution (40 Gm/100 ml distilled water). Trypsin — Trypsin D Novo Copenhagen Denmark. Haemoglobin — prepared in haemolyzing equine washed red blood cells and freeze-drying the clear supernatant after removal of the cell mem-

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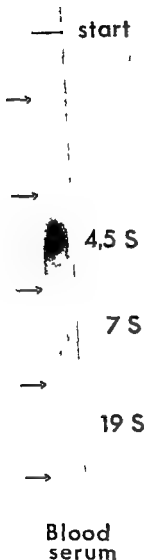


Figure 3 Stained filter paper strip with indications for cutting

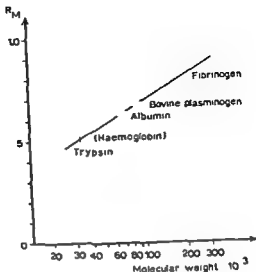


Figure 4 Curve obtained by plotting molecular weights of purified proteins against their R_M values Haemoglobin has an R_M value corresponding to a molecular weight of about 33 000

and directly stained for about 20 minutes in the Amido Black solution. The excess stain is removed by repeated washings in the acetic acid water methanol solution. For the last wash the strips are preferably left overnight in the solution. After they are dried at room temperature the colored fractions are cut out and eluted. The three protein fractions and a blank value are obtained by cutting pieces of the same size from the paper (Figure 3). They are eluted in glass stoppered test tubes by intermittent mixing for at least one hour. When normal serum is analyzed 40 ml of the eluting solution is used for the 4.5 S fraction and 10 ml for the others. Absorbance of the protein fractions is read against the blank in semimicrocuvettes with a 10 mm pathway at 620 nm and the percentage of each fraction is determined from the amount of stain. In calculating the approximate molecular size of a protein fraction, the migration distance from the starting point to the center of the spot (d_p) is divided by the distance traveled simultaneously by the macroglobulins (d_M)

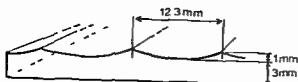


Figure 1 Cross section of glass plate for gel thin layer maximum depth of grooves = 10 mm

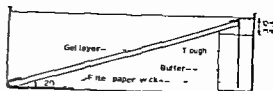


Figure 2 Vertical section of developing chamber Gel is layered on plate which is mounted as shown at an angle of about 20°. Lower filter paper wick ensures more even flow

brines by centrifugation Albumin — bovine crystalline serum albumin Armour Pharmaceutical Co. Kankakee, Illinois U.S.A. Bovine Plasminogen — prepared by the author's method (2) Fibrinogen — bovine fraction I 4 99% clottable prepared by the Blombäck's method (4)

Reagents Buffer consisting of 0.05 M tris and 1 M sodium chloride (pH adjusted to 8.0 with hydrochloric acid) was used throughout the procedure. **Staining Solution for Paper Strips** — 0.5 Gm Amido Black 10 B in 10 ml glacial acetic acid 40 ml distilled water and 50 ml methanol. **Wash Solution** — 10 ml glacial acetic acid 40 ml distilled water and 50 ml methanol. **Eluting Solution** — 0.5 Gm Na₂EDTA 1 M NaOH (500 ml) and 95% ethanol (500 ml)

Filter Paper Whatman No 3 MM

Transparent Plastic Box A bread box 7.5 x 19.5 x 26 cm was used as a developing chamber with a glass trough about 4 x 5 x 11 cm to hold the buffer (Figure 2)

Method

For most materials all operations can be carried out at room temperature. In a filtering flask 40 Gm of Sephadex is mixed with 100 ml of buffer. After the gel has been allowed to swell for 72 hours the entrapped air bubbles are removed by applying a vacuum (water suction pump) for about 15 minutes. The Sephadex gel is poured onto the clean dry glass plate placed on a table and distributed evenly in the grooves with a thin smooth edged glass spreader about 5 x 15 cm. The final layering is done by gently pulling the spreader lengthwise over the entire plate from one end of the glass to a point beyond

the other end. After 3 to 5 minutes the gel plate is suspended in a transparent plastic box (a level is used to ensure a perfectly flat surface) and equilibrated for at least 12 hours. The plate arrangement is shown in Figure 2. A wick of triple folded filter paper which sucks the buffer up onto the plate should extend about 10 mm onto the gel layer. At the start of the chromatography the top of the wick should be 10–15 mm above the buffer level in the trough to provide a suitable flow rate. The glass trough contains 150–200 ml of buffer. Slightly less than 20 µl of test solution (20 mg/ml) or undiluted serum is carefully deposited in the middle of each groove with a 20 µl semiconstriction micro pipette 20 mm from the plate's upper edge. It is important to avoid scratching the gel layer or blowing air through the pipette. Blue Dextran 2000 applied to one of the grooves acts as an indicator of the flow rate. The blue spot can easily be seen through the transparent box lid against a white background.

Development requires about 5 hours. The chromatography is considered complete when the Blue Dextran spot is observed about 40 cm from the lower end of the plate. The filter paper wick is then carefully removed and the gel plate is placed on a flat glass surface for 3 to 4 minutes. The grooves are covered with previously prepared strips of filter paper 10 x 300 mm which are rounded horizontally with the fingers to facilitate progressive adhesion from the centerline of each groove to the remaining gel surface. After the filter paper strips are allowed to absorb the solution from the gel for 5 minutes they are removed

Discussion

The thin layer gel filtration technique described here is used to separate proteins according to molecular size (volume). The contribution of lipoproteins to the normal macroglobulin value has been disregarded for this reason and because of certain technical details the results cannot be directly compared with ultracentrifuge analyses. This quantitative method however provides a simple means of estimating the amount of macroglobulins in a patient's serum. The mean value found for macroglobulins was 3.7 % and the coefficient of variance was 0.31 — relatively high compared to 0.04 for the albumin group. In none of the normal sera however did the macroglobulin value exceed 10 % of the total serum protein (duplicate analyses).

The technique is clinically useful to determine whether an M component detected by paper electrophoresis consists of macroglobulins or 7 S gamma globulins. The method has also been used for comparative study of serum proteins from human lymph and blood (3).

Summary

A thin layer gel filtration technique for proteins is described. It requires no special drying equipment and permits determination of the molecular size of purified protein fractions. This quantitative method has been used to determine normal values for human serum protein fractions. It is suggested as a valuable complement to paper and immuno electrophoresis in clinical applications.

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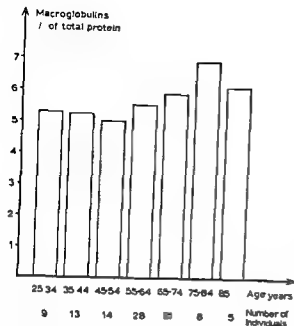


Figure 5 Macroglobulin values for different age groups

in normal human serum. A standard curve is prepared by plotting the ratio ($R_x = d\rho/d\rho_0$) for proteins with a known molecular weight (Figure 4).

Results

Results were not affected by the use of serum samples kept at -20°C up to one month. Reproducibility of the method was tested by analyzing one sample of normal human serum 12 times (Table 1). Normal values for the three groups of proteins in human serum were obtained by running two analyses on serum from each of 102 subjects — a total of 204 on which the calculations were based. Total protein values and results of paper electrophoresis in these persons were in the normal range. The mean values, standard deviations and approximate normal limits ($M \pm 2s$) for the

Table 1 A single serum sample analyzed twelve times: mean, standard deviation, and coefficient of variance are calculated for each protein group

Group of proteins	4 S	7 S	19 S
Mean %	69.6	25.0	5.4
Standard deviation	2.8	2.1	1.7
Coefficient of variance	0.04	0.11	0.31

Table 2 Mean values, standard deviations and approximate normal limits for practical use (about $M \pm 2s$) are calculated from 204 separate determinations (two analyses on each of 102 different normal serum samples)

Group of proteins	4 S	7 S	19 S
Mean (M) %	69.7	24.6	5.4
Standard deviation (s)	2.4	4.8	2.0
Approximate normal limits	60—80	15—35	<10

different fractions are shown in Table 2. The 19 S group of proteins did not account for more than 10 % of the total protein in any of the normal sera. When the macroglobulin values were grouped according to the subjects' ages, the means varied between 5 and 7 % with the higher values in the older age group (Figure 5).

Some purified proteins were found to give a straight line when the R_x values were plotted against the log for their respective molecular weights (Figure 4). Hemoglobin behaves differently, showing a molecular weight of only about 33,000 — a finding reported by several other investigators using similar methods (8).

cultures of peripheral blood or bone marrow from patients with Macroglobulinemia Waldenström (2 3 9 10 11, 14 17 21) The occurrence of this large supernumerary chromosome seems to be limited to the cells of the lymphoplasmoreticular system — in other words — to the tissue which has undergone neoplastic changes *Ferguson and Mackay* (10) were unable to demonstrate this abnormal chromosome in fibroblast cultures of their patient Whereas this extrachromosome of group A was at first described only in patients with Morbus Waldenström it has now also been demonstrated in patients with multiple myeloma (24 25 26) It represents the most frequent and outstanding chromosomal finding in paraproteinemias Today a distinct probability exists of a pathogenic relationship between this striking extrachromosome and the development of these neoplasms *Waldenström* (30) has indicated that with the acceptance of *Burnet's* clonal conception one must postulate that the responsible genetic make up of the individual is either present from birth through inheritance or is acquired through mutation in later life The question is important whether the extrachromosome of group A seen in patients with Morbus Waldenström or myeloma represents a specific abnormality or is the consequence of a mutant gene which leads to mitotic errors but has no connection with the neoplastic disease

In the course of family examinations among blood relatives of patients with Macroglobulinemia Waldenström

myeloma, we have found one case which may help clarify the above problem

Case report

Our patient Margrit K. born in 1906 was diagnosed as having Macroglobulinemia Waldenström in 1952 The 13 year course has been extremely benign and the general condition of this woman has remained relatively stable throughout this long time of observation The pathological findings are limited to the following: a moderate anemia of about 11 g% Hb an elevated sedimentation rate of 100/110 mm a pronounced γ M paraproteinemia (see fig 1 and 2) and cryoglobulinemia to which a large Ig L component seen in the ultracentrifugation corresponds characteristic changes of the bone marrow with infiltration with lymphocytes and large lymphoid reticulum cells as well as a slight increase in tissue mast cells Despite a marked decrease of the normal immune globulins only mild clinical symptoms due to antibody deficiency have become manifest The detailed findings of this case have been reported elsewhere (26)

Both parents are deceased there was no evidence of consanguinity All of the eight siblings of our patient are still alive including her twin sister In none of the five sisters and three brothers were we able by means of thorough electrophoretic and immunochemical examinations to substantiate any criteria for the existence of Morbus Waldenström myeloma or asymptomatic ("idiopathic") paraproteinemia This was also true in the case of our patient's twin sister

Genetic examinations

We have gone to great lengths to establish the possible monogenic relationship of our patient with paraproteinemias (Morbus Waldenström) and her healthy non paraproteinemias Information concerning the social membranes is not available due to the fact that the birth had taken place at

Chromosomal abnormalities in Macroglobulinemia Waldenstrom Discordant findings in uniovular twins

By G A SPENGLER,¹ H SIEBNER² and G RIVA

An increased familial incidence of Macroglobulinemia Waldenstrom or multiple myeloma has been repeatedly reported. Also an increase of other neoplastic diseases has been noted among the members of such families (27). Seligmann and co workers (23) are of the opinion that the incidence of paraproteinemia amongst blood relatives is higher than one would statistically expect in these relatively rare diseases (31). Therefore it would not be incongruous to postulate a genetic factor which may produce a hereditary pre-disposition to neoplastic changes such as those seen in the lymphoplasmatocellular system in cases of Morbus Waldenstrom, myeloma etc.

The pathogenesis of the paraproteinemic diseases is perhaps best explained by Burnet (4) who postulates a *mutation* as the basis of these neoplasms. Originally this mutation af-

flicts only a single cell but persists in the descendants of this cell "Burnet who thinks in terms of a bacteriologist compares this process with the growth of a bacterial colony in other words all plasmacytoma cells are descendants of a specifically different ancestor cell" (30). The same pathogenetic mechanism may also be considered valid for Morbus Waldenstrom. The theory of the monoclonal genesis of paraproteinemic diseases was accepted and enlarged upon by Waldenstrom. With his concept of *monoclonal hypergammaglobulinemia* (29, 30) he furthered the accepted principle that paraproteins are qualitative and/or quantitative abnormal variants of the immunoglobulins (13, 22). Through recent findings Burnet's postulate which bases the origin of paraproteinemic neoplasms on the mutation of a single cell of the lymphoplasmatocellular system has acquired a new aspect.

Numerous authors have demonstrated the presence of an *extrachromosomal* of group 1 in mitoses seen in

¹ Supported by a grant of the Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung

² Supported by a grant of the Deutsche Forschungsgemeinschaft

cultures of peripheral blood or bone marrow from patients with Macroglobulinemia Waldenström (2 3 9 10, 11 14 17 21) The occurrence of this large supernumerary chromosome seems to be limited to the cells of the lymphoplasmoreticular system — in other words — to the tissue which has undergone neoplastic changes Ferguson and Mackay (10) were unable to demonstrate this abnormal chromosome in fibroblast cultures of their patient Whereas this extrachromosome of group 4 was at first described only in patients with Morbus Waldenström it has now also been demonstrated in patients with multiple myeloma (24 25 26) It represents the most frequent and outstanding chromosomal finding in paraproteinemias Today a distinct probability exists of a pathogenetic relationship between this striking extrachromosomal and the development of these neoplasms Waldenström (30) has indicated that with the acceptance of Burnet's clonal conception one must postulate that the responsible genetic make up of the individual is either present from birth through inheritance or is acquired through mutation in later life The question is important whether the extrachromosome of group 4 seen in patients with Morbus Waldenström or myeloma represents a specific abnormality or is the consequence of a mutant gene which leads to mitotic errors but has no connection with the neoplastic disease

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Genetic examinations

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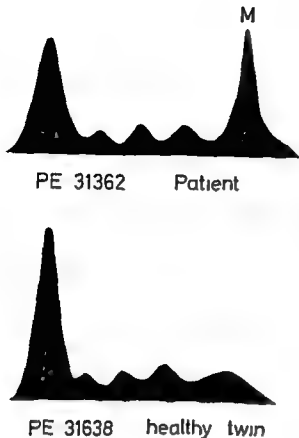


Fig 1 Electrophoretic serum protein patterns of the monozygotic twins

Upper diagram Our patient (Morbus Waldenström) with macroglobulinemia Waldenström shows a distinct M gradient (37 rel%) in γ position corresponding to the γ M paraproteinemias. Total serum protein 11.7 g% viscosity 6.4 ESR 100/110 mm

Lower diagram Her healthy uniovular twin sister (Johanna K.) shows an inconspicuous pattern. Total serum protein 6.7 g% viscosity 1.7 ESR 9/17 mm

home and the attending midwife has since died. Therefore we were dependent upon indirect methods.

The phenotype of the twin sisters has been remarkably similar since childhood and is even more outstanding when compared to that of the other siblings. General body frame, hair and eye colours as well as clothing and shoe sizes are identical. Inasmuch as both sisters are single and have no children corre-

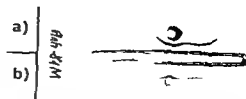


Fig 2 Immuno-electrophoretic findings in the monozygotic twins

a) Serum (undiluted) of our patient with γ M paraproteinemias (Morbus Waldenström) and anti γ M immunoglobulin antiserum. Only the accentuated γ M precipitation line demonstrating a double inflection and pronounced bends at both ends and parts of the macroglobulin fraction which have been precipitated round the serum deposit site have developed.

b) Serum (undiluted) of the healthy twin sister and anti γ M immunoglobulin antiserum. Only the normal γ M precipitation line has developed.

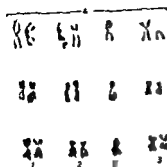
sponding examinations of descendants are out of question.

Blood and serum groups (ABO Rh CDE c^w MNS P₁ Kell Duffy haptoglobin Gc Gm Inv) were determined in all siblings. Whereas in the case of the twins all these factors are identical many variations exist among the other brothers and sisters.

Contrary to the rather common belief existing among both laymen and physicians *hand and fingerprint patterns* of uniovular siblings are by no means identical but may show great variations. Nevertheless fine details of the print patterns may be used to substantiate the fact of monozygosity. Appropriate criteria as defined by Maynard Smith and co-workers (16) were studied in our twin sisters.

The statistical evaluation of all our findings (blood and serum groups of all the siblings, hand and fingerprint patterns of the twin sisters) was carried out by Professor S. Rosin¹ and his co-worker Miss F. Schmid in a manner we greatly appreciated. It was

¹ Chief of the Division of Hereditary Research at the Zoological Institute of the University of Bern.



0 147
0 1 5
0 146

Fig. 3. Autosomal groups A of three metaphase plates with 47 chromosomes from our patient (Margrit) with Morbus Waldenström. The extra chromosome C lies between the autosomal pairs No. 2 and 3.

shown that the probability that the sisters were indeed monozygous existed with a certainty between 99.8190% (probability of error of this statement 0.1810%) and 99.999% (probability of error 0.0008%). The most probable value is 99.988% (probability of error 0.012%). With this we have the total assurance that our patient and her sister are undoubtedly twins. The pains taken to obtain this proof was necessary for the subsequent interpretation of our chromosomal studies undertaken in these two women.

In our patient Margrit with Morbus Waldenström a characteristic large extra chromosome of group A (6) was found in a portion of the metaphases of her blood and bone marrow cultures.

According to this size this additional chromosome lies between the autosomal pairs No. 2 and 3 and possesses a median to submedian centromere. The existence of an abnormal X chromosome was ruled out through the finding of no clonal sex chromatin patterns in the buccal smear and the presence of drumsticks in the leukocytes. Also the evaluation of the autoradiographic films showed no evidence of a supernumerary late labelling chromosome in addition to some metaphases the

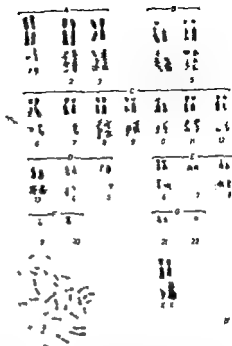


Fig. 4. Normal karyotype of the healthy twin sister (Johanna).

Upper rows: Chromosomes stained with hematoxylin.

Lower rows: Autoradiographic pattern (late phase) of the same chromosomes marked with the first labeled thymidine. Chromosomes stained with Giemsa.

chromosomes of group A demonstrated a stretch of the centromere region which was noticed in patients with Morbus Waldenström as well as in normal individuals (13, 24-26). Furthermore our patient — in addition to her cell line with the supernumerary chromosome of group A — also produced another cell line with 47 chromosomes. This line was characterized by a small almost metacentric extrachromosome of a size corresponding to the autosomes No. 20 (group F). As a rule one considers the presence of supernumerary small autosomes especially those of group G as a sign of increased malignancy in cases of neoplastic paraproteinemias (6, 26).

Table 1 Number of chromosomes in peripheral blood (72 hours of incubation) and bone marrow (7 hours of incubation) from the uniovular sisters (*italic numbers*=mitoses which were analyzed in detail with the aid of photographic mountings)

Number of chromosomes	<43	43	44	45	46	47	>47	Total of counted mitoses
<i>patient (Margrit A.)</i>								
blood culture	2	1	2	4	57	9	1	76
					19	9		28
marrow culture			1	2	15	4		22
					3	4		7
<i>healthy twin sister</i>								
1st blood culture				4	46			50
					17			17
2nd blood culture					10			10
					6			6

Despite this our patient has demonstrated a particularly benign course of Morbus Waldenström. In this case one could assume that the "trisomy F" originated from a cell with the large extrachromosome of group 4 which has lost the greater part of its long and short arms through deletion.

When cultures of peripheral blood from our patient were treated with tritiated thymidine the metaphase figures with 47 chromosomes showed either a very late stage of replicating DNA (with a "hot" X chromosome and a thin silvergrain over the autosomal set including the extrachromosome) or were not labelled at all. Thus in this patient we do not know the DNA replicating pattern of the extrachromosome up to now.

In the case of our patient's monozygotic twin sister who is clinically healthy and shows no abnormalities in her serum protein pattern repeated studies failed to reveal the presence of an extrachromosome of group A. Instead the evaluation of a total of sixty metaphases derived from two cultures of peripheral blood consistently showed a numerically normal chromosome complement. However this healthy twin did demonstrate an abnormal frequency of chromatid breakage. This mostly involved the autosome pair No 3 and could be noted in 24 to 30 % of the cells.

According to the findings of the Edinburgh group (7 & 28) stable and unstable aberrations may be found in 14 % of the cells of blood cultures taken from normal adults. This finding corresponds well with our experiences today on the basis of clinical and experimental observations. Viral infections of the patients or the culture media have been primarily incriminated as the cause of highly increased rates of chromatid breakage seen in blood cultures (18, 19, 20). At the time the first blood culture was drawn from the normal twin sister she was indeed suffering from an acute flu syndrome. It was for this reason that we repeated the examination four months later. Despite the fact that this second culture again showed increased breaks we would prefer not to interpret this as an inborn anomaly for such an increase in breaks was not present in the twin sister with Morbus Waldenström despite her tendency toward chromosomal abnormalities as demonstrated by her cell line with 47 chromosomes. We also do not believe that this increase of chromatid breaks results merely from adding tritium labelled thymidine inasmuch as they were also seen in unlabelled mitoses as well as in the control culture without tritiated thymidine.

Autoradiographs of ^3H thymidine marked

chromosomes showed a normal DNA replication pattern for the healthy twin sister with her numerically normal chromosomes (see fig 4)

Discussion

We have now followed a patient with typical Morbus Waldenström for 13 years. She demonstrates γ M paraproteinemia and characteristic bone marrow findings, whereas the clinical course to date has been extremely benign. In our opinion this case is unique and of particular interest inasmuch as she has a twin sister whom we have been able to examine thoroughly. Contrary to our patient this twin sister demonstrates none of the criteria for paraproteinemic disease and is clinically healthy. Statistical evaluation of data obtained from blood and serum group studies as well as palm and finger print examinations have shown these sisters to be of monozygotic origin with a certainty of 99.9788%. It follows that comparative chromosomal studies in this uniovular set of twins could be of paramount interest.

The fact that we could demonstrate a large extrachromosome of group A which was not of A chromosomal origin in the cultures from peripheral blood and bone marrow of our patient with Morbus Waldenström agrees well with the majority of the current literature reports as well as with our previous findings. In contrast to this careful evaluation of 60 metaphases from two separate blood cultures obtained from the healthy monozygotic twin failed to demonstrate the presence of such an

extrachromosome — the chromosome complement of this woman was numerically normal.

Inasmuch as monozygotic twins primarily possess identical genetic make up and therefore identical chromosomal patterns too one must assume that the extrachromosome of group A which was found only in the twin sister with Morbus Waldenström represents an acquired chromosomal mutation, a fact which is proved by the presence of a mosaic of normal/abnormal cells. Therefore it seems that a direct pathogenetic correlation exists between this acquired chromosomal mutation and the paraproteinemic neoplasm (Morbus Waldenström myeloma). The fact that many patients with macroglobulinemia or myeloma have demonstrated a similar chromosomal abnormality, namely an extra chromosome of group A, is in our opinion worth emphasis. For each individual patient this supernumerary chromosome represents a new acquisition which must be explained through a noxa effecting a strikingly similar aberration of the chromosomal complement. This aberration is apparently seen only in the tissue that has undergone neoplastic degeneration; in other words in cells of the lymphoplasmocytic system. As yet it is impossible to say whether either the paraprotein synthesis or the cellular neoplastic proliferation or both these phenomena together are dependent upon the presence of the additional chromosome of group A. If one supposes that in the increased familial incidence of paraproteinemic diseases

hereditary factors do indeed play a role, other modes of genetic transmission must be postulated, such as possibly through a recessive autosomal gene (23), perhaps predisposing to mitotic errors of lymphoplasmoretic cells.

In conclusion it should be noted that the findings herewith reported lie in the realm of rare natural experimentation. The findings also contain the pitfalls of all single observations. We would therefore like to call attention to the report of Goh and Swisher (12) which describes a male patient with chronic myelocytic leukemia in whom the typical Philadelphia chromosome was demonstrated. Here also a clinically healthy, monozygotic twin failed to demonstrate a chromosomal abnormality. This case which is most comparable to that of our twin sisters seems to substantiate our findings and conclusions.

Summary

A case is described concerning a female patient who has suffered for 13 years from Morbus Waldenström (benign form) with mild anemia, extremely increased ESR, γ M paraproteinemia (macroglobulinemia), and typical bone marrow changes. In cultures from her peripheral blood and bone marrow a large extrachromosome of group A could be demonstrated in a portion of the metaphases such as seen previously in other patients with paraproteinemic neoplasms. Our patient's twin — the monozygotic origin of the sisters could be statistically proved with a certainty

of 99.9788 % — is clinically healthy and shows no symptoms of a paraproteinemic disease. In two different blood cultures from the normal twin sister no extrachromosome could be demonstrated, and the chromosomal pattern was numerically normal. The extrachromosome of group A in our patient with Morbus Waldenström must therefore be regarded as an acquired chromosomal mutation. A direct pathogenetic correlation between the occurrence of this chromosomal aberration and the paraproteinemic disease seems to be proved.

Acknowledgements

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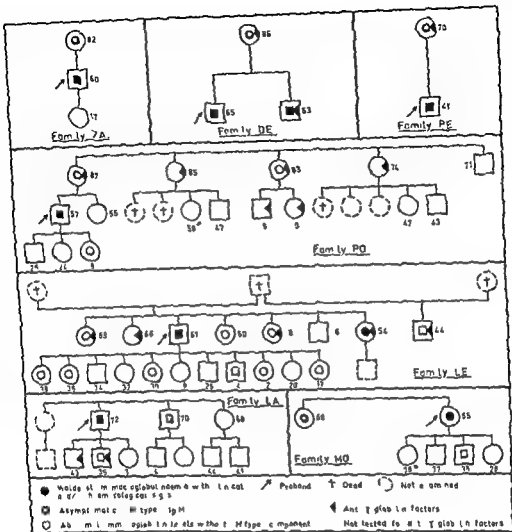


Fig 1

two and three years after the first serum examination the sharp IgM peak was no longer found on agar gel electrophoresis the ultracentrifugal patterns showed only 3% heavy components and IgM of both light chain monoclonic types were present (3). Chromosomal studies performed by J Lejeune and J Tanzer on most of

these patients and relatives with macroglobulinaemia have shown no large extra chromosome similar to that described by Bottura and al (4) except in a few cells of patient LA. Detailed clinical data on some of the probands can be found elsewhere (2) and (5) for ZA (1) for DE (6) for LE.

A comparative study of the macro

A Genetic Predisposition to Waldenström's Macroglobulinaemia

By MAXIME SELIGMANN

The finding of a familial occurrence of macroglobulinaemia in two families (1, 2) has stimulated an investigation of relatives of patients with Waldenström's macroglobulinaemia (WM). This systematic family survey has been accomplished with Drs François Daron, H Fudenberg and C Mihresco, and this paper gives a short summary of our results.

We have studied the sera of 216 close relatives (192 first degree) of 65 patients. This investigation is somewhat limited since in 35 families we were able to study only one or two first degree relatives. All the probands had the signs of WM with a lymphoid infiltration of the bone marrow and more than 25% heavy components on ultracentrifugation of the serum.

The three main abnormalities found in these families are: 1) presence of an Ig M type "M component" with or without evidence of disease; 2) abnormal immunoglobulin levels without

detectable "M component", 3) occurrence of anti- γ globulin factors in non elderly relatives.

The familial occurrence of Ig M type "M-components"

As shown in Fig 1, this has been found in 7 out of the 65 families. There was no evidence of consanguinity in these 7 families. Typical WM occurred in two siblings in each of two families. A significant increase of Ig M associated with a sharp band on electrophoresis has been found in 6 apparently healthy relatives: 4 mothers and 2 siblings. Clinical examination and blood counts were normal in these asymptomatic relatives. A sterneal puncture was performed in only one of them and showed no abnormality. The percentages of heavy components on ultracentrifugation of these 6 sera were respectively 6.9, 9.1, 12.1, 12.2 and 18%. The Ig G and Ig A levels were normal in four of these sera, significantly increased in one and greatly decreased in one. In the mother of family DE the Ig M "monoclonal" component was transient

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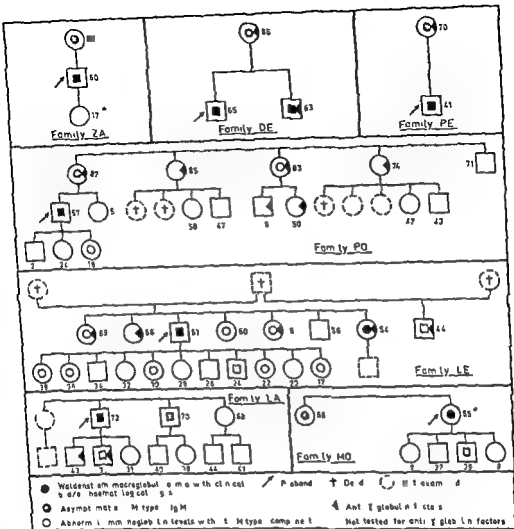


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Table 1 Abnormal immunoglobulin levels in first degree relatives of patients with Waldenstrom macroglobulinaemia

	No tested	High IgM type	High IgM			Low IgM			High IgA		Low IgA		Total abnormal	
			1	with high		1	with		1	with high IgG	1	with low IgG	No	%
				IgG	IgG and IgA		low IgA	high IgG and IgA						
Parents	18	4	1	1	—	2	1	1	2	—	—	1	13	72 ¹
Siblings	68	4	3	—	—	10	2	—	8	2	5	—	35	52 ¹
Offspring	106	—	6	1	3	6	4	—	5	—	10	1	37	35 ¹
Total	192	8	10	2	3	18	7	1	17	2	15	2	85	44 ¹

¹ Normal levels for the 2 other immunoglobulins

globulins in different affected members of the same family has been performed. Some differences have been found within the same family in electrophoretic mobilities, sedimentation constants, and Inv factors on the purified IgM. Starch gel electrophoresis of reduced and alkylated IgM showed that the mobility of the light chains was different in the affected members of the 3 families studied. The individual specific antigenic structure of the macroglobulins has been shown to be different in patient PE and his mother, in patient LC and his sister, in patient LA and his brother and in the two brothers DE. Studies with individual specific antisera to the IgM of each of these DE brothers have shown that their mother's serum contained two antigenically distinct macroglobulins, one with an antigenic structure similar to the macroglobulin of the first son and the other similar to that of the second son (7). No satisfactory explanation has been found for this peculiar finding.

Abnormal immunoglobulin levels without detectable "M component"

Immunoglobulin levels have been roughly estimated by immunoelectrophoretic analysis with different quantities of serum, and quantitative determinations have been performed by a method using radial diffusion in anti-body agar plates (8). The results obtained with random samples of a normal population have led us to consider as "abnormal" the following concentrations: for IgG < 7 mg/ml or > 18 mg/ml; for IgA < 1 mg/ml or > 3 mg/ml; for IgM < 0.6 mg/ml or > 2 mg/ml. Although we did not study matched control families, the overall incidence of immunoglobulin concentrations differing from the normal values is high in the first degree relatives of patients with WM (Table 1). Abnormal immunoglobulin levels have been found in 45 of the 65 families and in 24 of the 30 families which included more than 2 first degree relatives. As seen in Table 1, abnormal IgM and/or IgA levels are more fre-

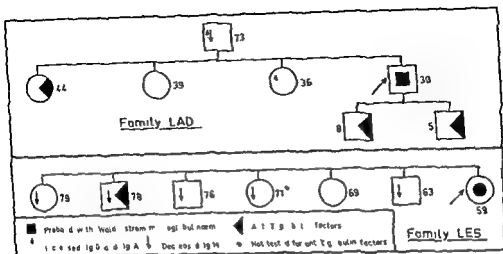


Fig 2

quent than abnormal IgG levels. Many different combinations of abnormalities are encountered (see for example in Fig 2 the father in family LAD). We have never found an important decrease of all 3 immunoglobulins nor an absence of any one immunoglobulin. Low IgM levels are more frequent than diffuse hyper IgM globulinaemia. In family LES (Fig 2) 5 of 6 siblings have a marked decrease of IgM. The high incidence of abnormal levels in the probands' parents must be emphasized. In siblings and offsprings the occurrence of abnormal immunoglobulin levels is independent of the sex. None of the offsprings with abnormal levels was less than 18 years old.

Anti γ globulin factors

The results of the tests with human and rabbit γ globulins are given in

Table II. Positive reactions have been found in 33 of 60 families and in 28 of 42 families with two or more studied relatives. The results are not related to the sex of the proband nor of the relatives. The incidence of positive reactions with either test in the elderly relatives is not significantly different from the findings in normal controls but the incidence is abnormally high in the relatives less than 60 years old. The two "sero positive" boys in family LAD (Fig 2) respectively 5 and 8 years old had never received transfusions nor γ globulin injections. There is no correlation between the IgM level and the positive serological reactions until γ globulin factors were found in only 7 of the 14 relatives with diffuse hyper IgM globulinaemia. The IgM level was normal in 80% of the sera with positive anti γ globulin reactions and low in 3 cases.

Table II Incidence of anti γ globulin factors in first degree relatives of patient with Waldenstrom macroglobulinemia

Age groups	<20	20-40	40-60	60-70	>70	Total relative
No tested	10	74	45	22	20	171
No positive reactions with						
Rabbit γ globulin	1	2	9	2	3	24
Human γ globulin ¹	1	9	14	3	6	33
No positive reactions with both tests	0	2	6	1	1	11
No of sera positive with either test	2 (20%)	10 (20%)	17 (38%)	4 (18%)	8 (40%)	46 (27%)

¹ = Ripley coat

Other antibodies

The results are essentially negative

Cold agglutinin titers have been measured in the sera of 157 relatives a titer of 1/16 was found in four sera and of 1/128 in two

Antinuclear factors have been investigated in 106 sera. The incidence of positive reactions was slightly higher than in a control group matched for sex and age but the difference was not significant and the titers were in general very low. Only 1 sera were positive at a titer of 1/50 and one serum had a titer of more than 1/100. This was from a 21 years old daughter of a WM patient. She showed no clinical abnormalities but her serum had very high levels of the three immunoglobulins and gave strongly positive anti human γ globulin reactions. Serological tests for syphilis were negative.

The incidence of anti thyroid and anti gastric autoan-

tibodies was lower than found in a control group

Discussion

Although the studies have been limited to a small number of relatives in many families they have shown a high incidence of quantitative and qualitative immunoglobulin abnormalities. Therefore the findings strongly suggest a genetic background in at least some cases of WM. Whether this genetic predisposition applies to all cases of WM or only to a subgroup within what may prove not to be a single disease is a matter of speculation.

An IgM type "M component" has been found in 8 of 216 relatives six of whom were apparently healthy. This is a high incidence compared to the 1% frequency of all types of M components in a Swedish normal population above 25 years old (9). All of these 8 relatives were more than 50 years old and 4 of the 14 still alive

probands' mothers showed an asymptomatic "monoclonal" Ig M component. The frequency of so called "essential M components" is known to increase with age (10). However in a control group of 100 healthy French women above 70 years old we have found only one Ig G type and no Ig M type "M components" and Halpern reports only one Ig M type "M component" in his series of 294 healthy people above 70 years old. The globulin abnormalities seems to become apparent only late in life in genetically predisposed individuals suggesting the influence of ageing and possibly of triggering environmental factors. We do not know if the relatives with asymptomatic macroglobulinaemia will later develop the progressive malignant disease. Some of these apparently healthy relatives have a rather high percentage of macroglobulin but on the other hand the "M component" has spontaneously disappeared in one mother. The sharp distinction between "benign essential monoclonal hyperglobulinaemia" and multiple myeloma (11) probably does not hold for microglobulinaemia since as Waldenström we have observed transitional forms.

The nature of the genetically determined disorder(s) cannot be deduced from the available data. The hypothesis of a structural gene abnormality is ruled out by the demonstration of a different biochemical and antigenic structure of the macroglobulin in affected members of the same family and besides there is no proof that Ig M of WM are truly abnormal mo-

lecules. Moreover the familial immunoglobulin abnormalities are not restricted to Ig M. In this family study we have found no Ig G or Ig A type "M components" but this has been observed by Halff and Hujmans (12) in a similar systematic family survey and amongst the recently reported cases with familial incidence of essential M components Ig G and Ig M types occurred within a same family (9). The variability of immunoglobulin abnormalities may indicate either that there is more than a single gene abnormality or that we are dealing with a basic disturbance of the homeostatic control of immunoglobulin synthesis and/or immune response.

A few observations of familial occurrence of multiple myeloma (13-14, 16-17, 18) and cryoglobulinaemia (19-20) have been published. The importance of both genetic and environmental factors in the mice plasma cell tumors is well recognized. The abnormalities observed in the families of patients with so called "acquired" primary agammaglobulinaemia (21-22, 23-24, 25) are very similar to our findings in WM families and lymphoreticular proliferation in some instances of malignant nature does occur in these patients. The hypothesis of a close analogy between "acquired" agammaglobulinaemias and macroglobulinaemias could therefore be suggested.

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Paraproteinaemia, Bence Jones Proteinuria and Amyloidosis

A clinical Study

By E. STUDER WOBMAN and F. HOLLER

Some years ago at the Congress of the German Society of Internal Medicine in Wiesbaden Jan Waldenström pointed out that although diagnostic methods become more and more complicated the recognition of macroglobulinaemia might be greatly facilitated by a very simple procedure needing only the naked eye—the Sia test. At present a few relatively simple methods (not as simple as the Sia test!) which can be performed in any hospital laboratory and of which immunoelectrophoresis is the most rewarding allow the paraproteinaemias to be recognized and also differentiated with sufficient accuracy.

In our department an average of 6 paraproteinaemias was recognized yearly between 1960 to 1964. Only one macroglobulinaemia was found (by ultracentrifugation) in this 5 years series. In 1965 with the introduction of immunoelectrophoresis as a routine procedure 18 paraproteinaemias were diagnosed including 7 plasmocytomas, 3 macroglobulinaemias, 8 "essential" paraproteinaemias and 1 symptomatic macroglobu-

linaemia. Although more blood samples than previously were sent to us from outside the hospital in 1965 the increase in frequency is due primarily to improved diagnosis.

A summary of the pertinent findings of our cases is given in Table 1. A few tentative conclusions from this clinical study may be of some interest.

Material and Methods

The following methods were used in each case:

Paper Electrophoresis (staining with amido black B) *Immunelectrophoresis* [Micro method according to Scheidegger (32)] of serum and urine (the latter being concentrated by dialysis against polyethylene glycol up to 1:10 or exceptionally up to 1:100). Antisera: Equine antihuman serum specific antisera against gamma A, gamma M and gamma G globulins and against Bence Jones Protein Type I (IgH) and II (IgL) (from the Blood Transfusion Service Amsterdam, the Behringwerke Marburg Germany and the Highland Laboratories USA). Semiquantitative determination of immunoglobulins for the detection of a deficiency of antibodies with the Agar diffusion technique according to Ouchterlony (2).

Sternal Puncture the ultracentrifuge technique as employed only for confirmation of the results obtained by immunoelectrophoresis in the cases with macroglobulinaemia. There was always agreement between the two methods.

Results and Discussion

Of the 18 cases with paraproteinaemia observed in 1963 8 presented the characteristics of gamma G (=gamma 5S) 6 of gamma M (=gamma 1 M) 3 of gamma A (=gamma 1 A) and 1 of gamma μ (Bence Jones type) globulin.

The sedimentation rate was markedly increased in 14 out of the 18 cases slightly increased in 2 and normal in 2. The total protein concentration in serum was increased only in 7 patients out of 18 in 2 cases it was even slightly decreased.

Examination of the sternal marrow allowed the diagnosis of plasmocytoma to be made in 4 out of 8 cases with gamma G in 2 of 3 cases with gamma A as well as in the case of gamma μ paraproteinaemia in other words in almost half of the cases (in 1 out of 17) there was no correlation between the immunoelectrophoretic and the morphological findings. The cases may be classified as "essential" or cryptogenic paraproteinaemias. Our observations give no evidence that they have in fact to be considered as plasmocytoma (Waldenström) but





























this possibility can by no means be excluded. It appears that in the early stages of the development of plasmocytoma the immunoelectrophoretic findings is more reliable than the result of the sternal puncture a statement which will be accepted only with reluctance by classical haematology. However several observations point in this direction (Osserman etc) — On the other hand we observed one patient with primary amyloidosis (confirmed by biopsy) who developed an unequivocal plasmocytoma (sternal puncture) during our observation and who did not present any of the characteristics of paraproteinaemia. Weicker has reported a similar case with plasmocytoma (without amyloidosis).

Therefore we have to admit that paraproteinaemia (of the gamma G and A type) may occur without plasmocytoma and that conversely plasmocytoma may in rare instances exist without paraproteinaemia.

Of the 6 cases with macroglobulinaemia 5 presented the typical lymphoid reticulum cells in the sternal marrow. Here again classical haematology is inclined to consider marrow morphology as more reliable than the abnormality of immunoglobulins. However if the cytological variability is taken into account the immunoelectrophoretic results appear definitely more accurate.

Legend: A Age BSR Blood Sedimentation Rate (mm in the 1st and 2nd hour) TP Total Protein Serum gr% IE=Immunoelectrophoresis M Grad M Gradient in gr% IL Inmunoelectrophoresis IL Ultracentrifuge MG Macroglobulins UP=Urinary Proteins BJ Bence Jones Urinary Type I & II Lymphoid RC Lymphoid Reticulum cells P Hyer pla = Plasma cellular hyperplasia Ne=Nephropathy β L po = β -Lipoproteins (normal values 1.660 g/dl)

Tab 1 Synopsis of the Findings in 18 Patients with Paraproteinemia

No	Age	BSR	IP	PE	VI Grad	IE (Serum)	UC	UP	BJ	Sternal Puncture	X ray	No	Amplio- dosis	P I po	Complications	
1	68	120/133	91		$\beta_{2.4}$ $\beta_{3.1}$		—	Alb γ	—	Plasmocytoma	+	—	—	280	Diabetes Rheumat Arthritis	
2	66	90/120	69		$\beta_{4.6}$		—	BJ	II	Plasmocytoma	+	+	—	981	—	
3	69	150/160	65		$\beta_{2.3}$		MG Alb	α ₂ γ	I	Lymphoid RC	—	—	—	330	Hemorrhagic Disease Pancreatitis	
4	48	160/161	91		$\beta_{5.5}$		MG	BJ	II	Lymphoid RC	—	—	—	148	Hemorrhagic Disease Anemia Antibody deficiency Syndrome	
5	76	60/89	68		$\beta_{4.7}$		—	BJ	II	Plasmocytoma	—	—	+	202	Diabetes	
6	68	92/116	77		$\beta_{4.4}$		—	BJ	I	Plasmocytoma	—	(+)	—	496	Pemphigus	
7	62	90/110	71		$\beta_{4.8}$ $\beta_{5.1}$		MG	Alb α ₂ BJ	—	Lymphoid RC	—	(+)	—	—	—	—
8	67	112/110	108		$\beta_{5.1}$		MG	n	I	Lymphoid RC	—	—	—	—	—	—
9	51	70/111	95		$\beta_{5.2}$		MG	—	—	Lymphoid RC	—	—	—	—	—	—
10	61	50/70	83		$\beta_{2.7}$		—	—	—	normal	—	—	—	—	—	Pneumonia
11	72	62/22	71		$\beta_{4.8}$		—	—	—	normal	—	+	—	—	—	Bronchiectasis
12	78	63/79	62		$\beta_{5.0}$		n	—	—	normal	—	(+)	—	128	Diabetes Gold Agglutination Disease	
13	64	125/110	106		$\beta_{5.5}$		—	Alb α γ	—	Plasmocytoma	+	—	—	376	Thrombophlebitis	
14	60	27/4	70		$\beta_{4.7}$		—	—	—	normal	—	—	—	470	Diabetes	
15	62	20/11	67		$\beta_{4.8}$		—	Alb β γ	—	pH Hyperplasia	—	—	—	593	Glaucoronephritis	
16	68	91/12	96		$\beta_{5.2}$ $\beta_{5.6}$		—	—	—	—	—	—	—	—	—	—

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this possibility can by no means be excluded. It appears that in the early stages of the development of plasmocytoma the immunoelectrophoretic finding is more reliable than the result of the sternal puncture a statement which will be accepted only with reluctance by classical haematology. However several observations point in this direction (Osserman etc.) — On the other hand we observed one patient with primary amyloidosis (confirmed by biopsy) who developed an unequivocal plasmocytoma (sternal puncture) during our observation and who did not present any of the characteristics of paraproteinaemia. Weicker has reported a similar case with plasmocytoma (without amyloidosis).

Therefore we have to admit that paraproteinaemia (of the gamma G and A type) may occur without plasmocytoma and that conversely plasmocytoma may in rare instances exist without paraproteinaemia.

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I = Ig A, Ig E, BSR = Blood Sedimentation Rate (mm in the 1st and 2nd hour) TP = Total Protein in Serum gr%. IE = Paper Electrophoresis Vgrad = M Gradient in gr%. IE = Immunoelectrophoresis UC = Ultracentrifuge MC = Macroglobulins LI = Lary Proteins BJ = Bence Jones Protein Type I & II Lymphoid RC = Lymphoid Reticulum cells PI = Hyperplasia of Plasmacytoid cells perleas = Leukocytopathy β L₁ = β Lipoproteins (normal values 1.660-1.1)

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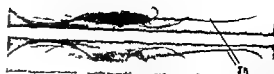
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Case 4

Conc 1 3



Precipitation with antihuman serum from the horse



Case 8

Conc 1 3



Case 9

Conc 1 3

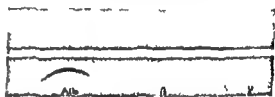
Fig 1 γ M Paraproteinemia Precipitation with equine antihuman serum

Whereas the quantity of abnormal globulins has apparently no direct prognostic significance in paraproteinemia in general it seems that the macroglobulinemias with exceptionally large globulins (which do not migrate in agar gel) present a particularly serious prognosis (cases 3 and 9)

In 1 case macroglobulinemia was found associated with cold agglutinins

Immunoelectrophoresis being the most reliable method for the detection of Bence Jones protein it is of interest to note that with this technique the BJ protein may be found in urine and serum of various types of paraproteinemia including macroglobu-

Specific precipitation with anti gamma macro globulin serum pathologic gamma M precipitation line



Conc 1 3

Identification of the uroproteins with anti human serum Precipitation lines of the albumin (Alb) beta globulin (β) and gamma globulin type (γ)



Conc 1 3

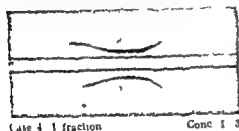
Specific precipitation of the micromolecular uroprotein with anti BJ serum type 1

Fig 2 Gamma M Paraproteinemia Waldenström case 7

linemia (Table 1 2 cases of gamma G 3 cases of gamma M and the gamma μ case) We confirm therefore the findings of Weicker and others who showed that Bence Jones protein



Case 9 2 fractions with immunologically different precipitation the first does not migrate in agar gel



Case 4 1 fraction



Case 11 2 to 3 various fractions Conc 1 3

Fig 3 Various types of gamma M paraproteinemia (macroglobulinemia) Specific precipitation with equine anti gamma M serum

can occur in all types of paraproteinemia.

However this low molecular protein can — even with immunoelectrophoretic technique — not been detected in all cases of paraproteinemia (Table 1). Therefore if Kahler's merit of having correlated Bence Jones protein with multiple myeloma subsists it has to be stressed that the absence of this protein rules out plasmocytoma just as

little as its presence excludes macroglobulinaemia.

The relationship between amyloidosis and abnormal gammaglobulins was first suggested by Magnus Levy in 1931 who emphasized the frequent occurrence of Bence Jones protein in this condition. Aptiz who found abnormal plasma cells in cases of primary amyloidosis designated this disease as "premyeloma". Osseman in a careful study presented evidence that the amyloid infiltrates are formed by aggregates of gammaglobulin fragments of the Bence Jones type with tissue proteins and/or polysaccharides. One of our cases with gamma G paraproteinemia and plasmocytoma presented amyloid deposits in the spleen and liver at autopsy. We have already mentioned the case with very pronounced primary amyloidosis (involving the tongue, the muscles of the gastrointestinal tract, the heart and the kidney) who developed a plasmocytoma during the 9 month of our observation. No evidence for paraproteinemia (or Bence Jones protein) could be found in this patient.

The problem of the development of amyloidosis is far from being solved. But the relationship to abnormal immunoglobulins produced by abnormal plasma cells casts some light on the pathogenesis of primary amyloidosis which up to now has been entirely enigmatic.

Summary

Immunoelectrophoresis which can be performed in any hospital laboratory

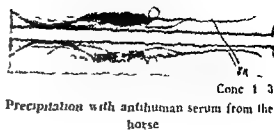


Fig 1 γ M Paraproteinemia Precipitation with equine antihuman serum

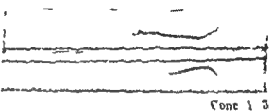
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In 1 case macroglobulinemia was found associated with cold agglutinins.

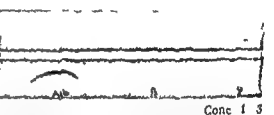
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Precipitation with antihuman serum from the horse



Specific precipitation with anti gamma macro globulin serum pathologic gamma M precipitation line



Identification of the uroproteins with anti human serum Precipitation lines of the albumin (Alb) beta globulin (β) and gamma globulin type (γ)



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is of value in the diagnosis and differentiation of paraproteinemias in so far as it is probably superior to serum puncture in the early recognition of plasmacytoma. Immunoelectrophoresis is also the most reliable method for the detection of Bence Jones protein, which may be found in any type of paraproteinemia. Primary amyloidosis may precede or accompany plasmacytoma.

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Fig. 1 Bone marrow smear (sternal puncture on admission). Note the typical dense infiltration of the marrow with small lymphoid cells, plasma cells and some tissue mast cells. The normal marrow elements are scarce.

Sternal marrow There was a severe infiltration of the marrow (Fig. 1) with small lymphoid reticulum cells. Some of these cells had a small border of surrounding protoplasm of bluish aspect. There was a distinct increase of the plasmacellular reticulum cells and the tissue mast cells. The erythropoiesis was quantitatively diminished and normoblastic. There was a normal activity of the granulopoiesis and there was a normal amount of megacaryocytes.

Chest x ray on admission (Fig. 2a). Diffuse small nodular infiltrations which are confluent in some parts of the lung. The x ray of the pelvis shows slight diffuse osteoporosis but no osteolytic foci.

Special examinations for paraproteins Sia test positive total protein 4.6 g%, A/G ratio 0.47 alb 31.8 rel.%, α_1 glob 0.3 rel.%, α_2 globulin 9.4 rel.%, β globulin 9.7 rel.%, γ globulin 29.1 rel.%, and γ globulin 14.7 rel.%, viscosity 2.00 to 2.80. Immuno electrophoresis (serum) paraproteinemia of the γ M globulin type. Electrophoresis of the 24 hour urine. The slight amount of protein present in the urine almost entirely consisted of the γ M type. In the ultracentrifugation of the serum we found an abnormal macroglobulin with a sedimentation constant S of 14 Svedberg units quantitatively they made up about 1.5% of the total protein.

ha 17.1% monocytes 9.5% and lymphocytes 2.4%. The erythrocyte count was 4 mill per mm³. The erythrocytes were mainly normochromic normocytic there was microcytosis.

In the urine there was a trace of albumin and there was no Bence Jones proteinuria. The 1100 urea was 31 mg% latex test 1 (trist) < normal. Leukocyte alkaline phosphatase 111 normal 90 to 500. Vital capacity 1 litre (normal 2.5). All tests for tuberculosis negative. Microscopically and in the sputum culture no tubercle bacilli. Mantoux test at a dilution of 1:1000. Sputum examination for fungus were negative. Complement reactions for histoplasmosis and cryptococcosis were negative.

Biopsies

Lymphnode (Biopsy fecit PD Dr R Berchold Histology Institute of pathology Aarau Dr I P Muhlethaler). The normal structure of the lymphnodes was almost completely destroyed. There were still some nodules which contained small lymphoid cells. capsule of the nodes was infiltrated with small lymphoid cells. Diagnosis malignant lymphoma of the nodular lymphocytic type.

The liver biopsy showed a normal histological picture.

Lung biopsy (operation by PD Dr R Berchold). A piece of 3/2.08 cm gray brownish lung was excised. Histology (Institute of pathology Aarau, Dr I P Muhlethaler). In

Macroglobulinemia Waldenstrom with milary lunginfiltrations and terminal plasmacell-leukemia¹

(Chlorambucil induced clinical remission despite autoradiographically
persistant DNA- and RNA synthesis)

By SVEN MOESCHLIN

Introduction My dear friend Jan Waldenstrom has described several new syndroms in the field of internal medicine. Despite the modern tendency of overspecialisation, he always remained the big master in both the wide field of general internal medicine and hematology as well. Because of his special interest in hematology I would like report a case of a syndrome in this field which bears his own name. We have partly described this case before (1), but its further clinical course and the terminal transition into plasmacell leukemia is of a special interest.

Case history GE 79 year old woman admitted to the hospital on March 2 1963 with generalized adenopathy and milary lunginfiltrations with severe cachexia.

The disease started in the beginning of 1961 with increasing fatigue and general weakness. In summer 1961 an enlarged submandibular lymphnode was noted on the left

side. Since then there was continuous anorexia, often nausea and emesis and progressive loss of weight, totally 13.5 kg. In July 1962 generalized lymphadenopathy was noted and for the first time a milary lung picture was seen. The sedimentation rate was increased to 62 mm in the first hour. Histologically an excised submandibular node showed a picture resembling a follicular lymphoblastoma of moderate cell size. Since the end of 1962 there was recurrent epistaxis, dyspnea, cough and progressive cachexia.

Clinical findings On the day of admission (March 2 1963) she was thin and pale and had a generalized lymphadenopathy. Some of the nodes had the size of a medium plum and they were found in the submandibular, cervical, supraclavicular, axillar and inguinal area. The liver was not enlarged; the spleen measured 10 1/2 cm but was not palpable. On auscultation of the lungs and the heart there were no abnormal findings. The blood pressure was 140/86, the pulse 96. No infiltrations in the ocular fundus.

Laboratory findings on admission Sedimentation rate 130/135 mm (20° C) and 129/134 mm (37° C). Hemoglobin 12.4 g%, hematocrit 38%, reticulocytes 6‰, thrombocytes 300 000, leukocytes 4 800 with the following differential: Juvenile neutrophils 4%, segmented neutrophils 46%, eosinophils 1.4%,

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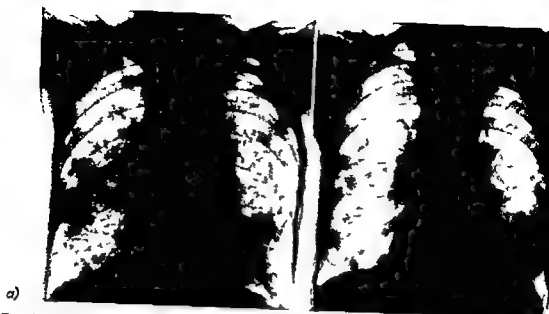


Fig 2 a) Chest x ray on admission March 1963. Note the diffuse pulmonary infiltrations which are confluent in some parts of the lungs.
b) Striking improvement of the lung picture after two years of treatment with Chlorambucil 6–10 mg daily combined with prednisone 30 mg daily.

teralveolar and especially interlobular septa as well as the peribronchial and perivascular fibrous tissue was thickened and infiltrated with cells. These infiltrations consisted of lymphocytes and plasma cells and often also of small cells without cytoplasm. These cells were also seen intravascularly. The cell elements seen in this histological picture resembled the cells seen in Waldenström's disease (Fig 3).

Therapy and course Before the lung biopsy had been performed treatment with different antibiotics and tuberculostatics showed no result. The lung infiltrations persisted and even increased. After cytostatic therapy was begun with Chlorambucil (Leukcran) with an initial dose of 6 mg daily and later 10 mg daily combined with prednisone 30 mg daily (Fig 4) there was considerable improvement. There was a weight gain of 6 kg. The general adenopathy decreased and the paraproteinemia slowly returned to normal. After some 2½ months of therapy on May 27 1963 no paraproteins could be detected in the serum by paper electrophoresis and immunoelectrophoresis. The improvement of the lung in-

filtrations and the sternal marrow picture however was much slower. Also there was persistent paraproteinuria which was still present after almost 4 months of treatment.

Autoradiographic studies of DNA and the RNA-synthesis Methocel Aspirated marrow was incubated 1 hour in siliconised tubes at 37°C with H^3 thymidine, H^3 uridine and H^3 cytidine (further details see (1)). These studies showed that the H^3 thymidine (DNA) was localized in the nucleus. The same held true for H^3 uridine and H^3 cytidine after 1 hour of incubation. Only after an incubation of over 2 hours H^3 uridine and H^3 cytosine which are both incorporated into RNA had migrated from the nucleus into the cytoplasm.

Further course (Fig. 4) The patient was discharged from the hospital on July 2 1963. At home she continued to take 4 mg of Chlor-

after stopping cytostatic treatment. The patient was controlled on an ambulatory basis. She remained well until September 13, 1963. Afterwards the general condition markedly deteriorated. There were distinct edema and enlarged lymph nodes in the mandibular submandibular cervical and axillary area. The liver was markedly enlarged and could be felt at the right costal margin. The spleen was palpable 3 cm under the left costal margin. There were petechiae on the forearms and the conjunctivae. Body weight 49 kg.

Reexamination of the signs of Waldenström's disease

a. *Skeletal marrow* Now again there was a distinct increase of young small lymphoid cells and of plasmocellular reticulum cells. Some of the plasmacells had distinct nucleoli in the large nucleus.

b. *Lung picture* The infiltrations of the lung have increased again and there are signs of pleuritis on both sides.

Laboratory findings Sedimentation rate 14-39 mm, hemoglobin 7.5 g%, leukocytes 2600 per mm³ with normal differential count, thrombocytes 20000 per mm³. Paper electrophoresis and immunoelectrophoresis of the serum: hypoproteinaemia, increased gamma globulin. The interpretation of the gamma immunoglobulin preparation line was difficult and is not distinct enough to diagnose a gamma M immunoglobulin paraproteinoma. The electrophoresis: paraproteinoma with a large M-globulin in the β - γ globulin region. This was essentially the same finding as in the year 1964. The immunoelectrophoresis of the urine gave no distinct finding but looks suspicious for a paraproteinoma of the Ben-Jones type. Liver function of the serum: In conclusion the previous findings in 1964, no clinical neurological signs present.

Two weeks after the last ambulatory control the patient reenters the hospital on September 18 in an emergency case. There was a cerebral hemorrhage, dizziness with ataxial tremor and profuse bleeding from the mouth although the patient had been off

Chlorambucil since February 1963. The hemoglobin was 1 g%, the leukocyte count 1900 per mm³, prothrombin activity (Quick) 20%, Urea 184 mg%. The patient died despite of blood transfusions, Epsamone, Cortisone intravenously etc.

Blood smear In the differential leukocyte count there were 30% plasma cells of distinct pathological aspect (see Fig. 3a). The cells exhibited a very pronounced polymorphism. There were smaller forms and very large forms but all these plasma cells showed very large and sometimes up to 2-3 nucleoli in a large nucleus. The cell type is very similar to the blast forms of the pathological plasma cells as seen in other cases of plasma cell leukemia.

Sternal marrow The marrow was now completely infiltrated with the same polymorphic undifferentiated big plasma cells with a fine chromatin structure of the nucleus and 2 to 3 very sharp large nucleoli (Fig. 3b). The nucleus was very large compared to the small border of deep blue cytoplasm. There were very few eosinophilic granulocytes and lymphoid reticulum cells left.

Diagnosis Terminal acute plasma cell leukemia with complete infiltration of the bone marrow; secondary thrombocytopenia with severe hemorrhages causing the death of the patient on September 16, 1963.

Autopsy (Institute of Pathology, Kantonspital, Zurich, Dr. I. M. Mühlethaler). Dissection of all lymph nodes, the pericardium, myocardium and of the lungs with secondary amyloidosis of the lungs. Plasmocellular infiltrations of the spleen, liver, kidneys and the adrenal glands. Infiltration of the bone marrow as this is seen in terminal plasma cell leukemia. In addition there was an adenocarcinoma of the rectum with bleeding into the intestinal tract and bilateral hypostatic bronchopneumonia.

Discussion

This case of Waldenström's disease is of special interest from different points of view. A woman of 49 years

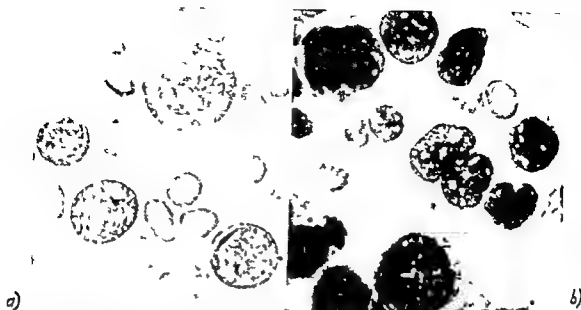


Fig 5 a) Plasma cells of acute plasma cell leukemia in the peripheral blood smear. They exhibit a pronounced polymorphism with large nucleoli.
b) The sternal marrow was infiltrated with the same polymorphic undifferentiated big plasma cells. Only very few marrow elements were left.

20, hemoglobin 12.6 g%, leukocytes 3,600 with normal differential count, thrombocytes 45,000, probably due to the constant cytostatic therapy. Serum electrophoresis and immunoelectrophoresis: no findings whatsoever for a paraproteinemia. The sedimentation rate was normal, which was in sharp contrast to the one on initial admission. Urine electrophoresis: trace of protein; again there was a small paraprotein gradient in the β - α globulin region.

The patient was dismissed from the hospital on June 26, 1963. Her body weight at that time was 45 kg. Therapeutically she continued on 4 mg of Chlorambucil daily (Fig 4). At home she felt well in the beginning. In fall 1963 she slowly deteriorated and was again hospitalized on December 23, 1963. She remained in hospital until February 18, 1964. On this third admission she was febrile and had a bilateral bronchopneumonia. The sedimentation rate was again very high (131/141), leukocytes 12,300 with a distinct shift to the left of the neutrophils and toxic granulations. After an antibiotic treatment the pneumonic infiltrations disappeared and the general condition improved.

Sternal marrow Now completely normal. No findings whatsoever of Waldenström's disease any more.

Pulmonary x-ray After disappearance of the bronchopneumonic infiltrations on admission, also a further regression of the previous specific infiltrations of Waldenström could be detected (see Fig 2b).

Laboratory findings: Sedimentation rate 15, hemoglobin 9 g%, urine no albumine, leukocytes 3,000, thrombocytes 20–30,000. Serum electrophoresis and immunoelectrophoresis: no trace of protein anymore. Ultra-centrifugation: no pathological macroglobulin present anymore (in contrast to the findings in 1963!).

Final course Because of the leukopenia and thrombocytopenia, Chlorambucil therapy was stopped in February 1964. Stopping treatment was further justified by the fact that the electrophoretic and bone marrow findings gave no evidence of active macroglobulinemia of Waldenström. Now it was of great interest to see if the previous findings of Waldenström's disease would slowly return.

urine disappeared completely and the bone marrow picture returned to normal. The same normalisation of the serum has been observed by Alleran treatment in other cases (9, 10).

The striking feature of this case is the final transition into an acute plasma cell leukemia.

It might be possible that the autopsy case described by Schamaun (3) also had terminal plasma cell leukemia. Here plasma cell infiltrations in nearly all organs were found. In our case it was very interesting to note that despite the final recurrence of plasma cell infiltrations of the marrow and the most other organs found at autopsy and despite of the terminal plasma cell leukemia there was only a slight recurrence of the paraprotein formation.

This in our opinion is probably due to the transition of these cells into very undifferentiated immature neoplastic cells in the terminal course of the disease. It must be assumed that most of these undifferentiated cells were not able to produce paraproteins any more. This can be compared in some way to the cessation of the normal production of hormones in very undifferentiated neoplastic cells for example in a carcinoma of the thyroid etc.

The third point which has to be emphasized in our patient is the occurrence of other malignant lesions. Waldenström personally has several times pointed out the strange coincidence of the occurrence of paraproteins and neoplastic transformation. Our patient developed two different

carcinomas first in 1963 a carcinoma of the upper lip (spinocellular type). This lesion could be cured by x-ray therapy. At autopsy a second carcinoma of different type (adenocarcinoma) of the cecum was found. So this patient developed two different carcinomas while she had Waldenström's disease. How this neoplastic transformation is related to Waldenström's disease is still unknown. Is it possible that the same "virus" once localizes in the reticuloendothelial system and other times in other cell types and causes them to proliferate? Another possibility is that there is a direct connection between paraproteins and malignant transformation. These are only questions which can be raised but not answered at the present time.

Summary

A case of Waldenström's disease with generalized adenopathy, typical bone marrow infiltration and bilateral lung infiltrations which could be proved by biopsy to be specific infiltrations of Waldenström's disease is reported.

Typical paraproteinemia and albuminuria of the γ M globulin type were present. The patient was treated continuously with Chlorambucil during a period of two years and showed a marked improvement. The sedimentation rate which was very high initially returned to normal after two years. The sternal marrow showed a complete remission with disappearance of the lymphoid and plasmocellular infiltrations and the serum paper and

was suffering from increasing fatigue, anorexia and loss of weight since 1961. In August 1961 she developed generalized progressive adenopathy and in July 1962 a milary lung picture. Clinically a typical Waldenström's disease with a sedimentation rate of 130 mm, a positive S₁₁ test and a paraprotein peak in the γ region on paper electrophoresis of 2.85 g% was found. Immunoelectrophoretically this paraprotein showed to be of the Gamma₁_x type. On ultracentrifugation it proved to be a microglobulin of the S₁₄ Svedberg type and it made up 15 % of the total proteins. Bence Jones proteinuria was not found but there was a distinct paraproteinuria which immunoelectrophoretically showed a distinct M gradient. The sternal marrow was infiltrated with typical lymphoid reticulum cells, plasma cells and tissue mast cells. Fine milary nodules of the lung proved to be histologically typical lymphoid and plasma cell infiltrations as seen in Waldenström's disease infiltrations in the bone marrow. Treatment with Chlorambucil induced an impressive remission during 4 months with a weight gain of 6 kg, normalization of the sedimentation rate and the serum paper electrophoresis and immuno electrophoresis and nearly complete disappearance of the generalized adenopathy. In the beginning paraproteinuria still persisted and the pathological infiltrations of the bone marrow and the lungs only partially regressed. After further treatment with 4 mg of Chlorambucil daily during one year a complete remission of the bone marrow and a nearly

complete remission of the pulmonary infiltrations as well as the adenopathy was induced. In 1964 the serum paper electrophoresis and immuno electrophoresis showed no signs of paraproteinemia any more and finally also the paraproteinuria disappeared. After stopping the Chlorambucil treatment (February 1965) the paraproteinuria reappeared in fall 1965.

In the literature one case of Waldenström's disease with lung infiltrations has been reported "case 8" by *Lurie* (2). A more diffuse milary type has been described for the first time in one case by *Schamaun* (3) in 1954. This was an autopsy finding and clinically the infiltrations had not been detected. (4) *Oettingen et al* (5) reported another case of milary infiltrations discovered at autopsy. In their case the lung infiltrations had remained clinically undetected. (6) *Noach* (7) for the first time in 1956 described a 56 year old patient with Waldenström's disease of the α_2 type with bilateral lung infiltrations. These lesions disappeared after x-ray treatment. Also in the cases of *Revol* (8) and *Lurie* (2) (case 2) one year before death a reticular milary lung picture could be demonstrated as in our case.

In our case the lung infiltrations could be demonstrated for the first time by biopsy in a living patient to be specific infiltrations with plasma cells and lymphoid cells of the Waldenström type. For the first time they also showed distinct regression of a two years continuous treatment with Chlorambucil. At the same time the paraproteins in the serum and in the

urine disappeared completely and the bone marrow picture returned to normal. The same normalization of the serum has been observed by Alkeran treatment in other cases (9, 10).

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What is Waldenström's Macroglobulinemia?

By WILLIAM DAMESBULA M D

Waldenström's macroglobulinemia may be said to be characterized chiefly by two features (1) a striking increase in γ M globulin of the "monoclonal gammopathy" type and (2) an abnormal bone marrow characterized by either a leukemic or leukemic like picture in which lymphocytes of various types are prominent (1 2 3 4). All the other features — hemorrhagic hemolytic rheologic (viscosity syndrome) must be considered as secondary manifestations. Is this condition to be considered a dysproteinemia i.e. a peculiar disturbance of protein production a form of chronic leukemia in which one of the immunoglobulins is produced in high concentrations or could it conceivably be an abnormal or aberrant (neoplastic) type of immunological response? Perhaps all of these possibilities are correct in one way or another and it is the purpose of this communication in

Honor of Jan Waldenström's 60th birthday to attempt a unifying hypothesis. To do this it is necessary to turn to some relatively basic considerations which involve the immunoglobulins and the immunocompetent cells (immunocytes), and to some clinical considerations regarding autoimmunity and what I have ventured to call the "immunoproliferative disorders" (5 6).

The first stage of the immune response is marked by the production of antibody of the macroglobulin variety following which antibody of the γ G type appears (7 8). It is of interest in this regard that phylogenetically, γ M is the primordial immunoglobulin and γ G a later development (9, 10 11). It is conceivable that the γ M and the γ G responses are quite separate although sequential and related through a "feedback" mechanism (cf below). Parallel studies of tissues during the immune response have indicated that the γ G phase of the response is associated with plasmacytosis the earlier cellular response to antigen has not

immuno electrophoresis showed no findings whatsoever for a paraprotein anymore. The pathological macroglobulin found by ultracentrifugation had also disappeared after treatment. In contrast to the typical findings in 1963, the lung infiltrations were considerably decreased after therapy, but the chest x ray did not return completely to normal. Autoradiographic studies of the DNA- and RNA-synthesis by incubation with H^3 thymidine, H^3 -uridine and H^3 cytidine still showed active DNA synthesis in the nucleus after one year of treatment. Only after incubation of at least two hours, H^3 -uridine and H^3 cytidine had migrated from a nucleus into the cytoplasm.

In the final course, the patient developed a typical plasma cell leukemia with 35 % plasma cell in the peripheral blood. The leukocyte count was 1,900 per mm³ at that time and all the organs were infiltrated with large polymorphic and undifferentiated plasma cells.

These findings were verified by autopsy and in addition a secondary amyloidosis of the lung was found.

The patient developed two different carcinomas during the course of her illness. One of them was on the upper lip, and was cured with x ray treatment. At autopsy, an additional carcinoma of different type, namely an adenocarcinoma of the cecum was found.

The different interesting aspects of this case are discussed.

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honor of Jan Waldenström's 60th birthday to attempt a unifying hypothesis. To do this it is necessary to turn to some relatively basic considerations which involve the immunoglobulins and the immunocompetent cells (immunocytes) and to some clinical considerations regarding autoimmunity and what I have ventured to call the "immunoproliferative disorders" (5 6).

The first stage of the immune response is marked by the production of antibody of the macroglobulin variety following which antibody of the γ G type appears (7 8). It is of interest in this regard that phylogenetically, γ M is the primordial immunoglobulin and γ G a later development (9 10 11). It is conceivable that the γ M and the γ G responses are quite separate although sequential and related through a "feedback" mechanism (cf. below). Parallel studies of tissues during the immune response have indicated that the γ G phase of the response is associated with plasmocytosis; the earlier cellular response to antigen has not

yet been clearly defined. It is clear, however, that foreign or "not-self" materials are phagocytized by macrophages (histiocytes, reticuloendothelial cells, probably the most primitive of the protective devices against foreign materials). These phagocytic cells probably digest and "simplify" the engulfed material so that its contained antigen or even a simpler substance with antigenic information can be brought into contact with cells more immediately concerned with the cellular immune response (12). It has been shown that small or "mature" lymphocytes gather about macrophages containing antigen, sending out pseudopods which may have intimate and prolonged contact with the macrophages (13, 14). Conceivably, the transfer of antigen or of simplified "information" to lymphocytes is made at this time, whence the actual induction of immunization may be said to begin. The lymphocytes then appear to "differentiate" to become large primitive looking cells with pyroninophilic cytoplasm, and variously called blast cells, hemocytoblasts, or, in my own vocabulary, "immunoblasts" (15). These primitive cells may divide and produce more lymphocytes. Whether they also produce plasmocytes is not completely certain although it seems very likely. Since the plasmocytes are certainly productive of γ G and A globulins, and are not clearly involved in γ M production, it would seem likely that the lymphocytes and perhaps the immunoblasts — which contain very large numbers of ribosomes and a primitive ergastoplasm — may be macro-

globulin producing units. However, attempts to define this by such techniques as immunofluorescence, cell cultures, etc., have for the most part been unsuccessful. Thus, the exact source of the macroglobulins remains ill defined. Not so with the 7 S globulin, which is clearly associated with the plasma cells (16, 17, 18). The exact derivation of the latter cells, whether from reticulum cells, lymphocytes, or from immunoblasts, remains at this time more obscure than ever, it is even possible that the plasmocyte is simply a modified lymphocyte in which the message for γ G protein (globulin) production has been received and is being acted upon, as indicated by the characteristic ergastoplasmic structure.

It has been demonstrated by Schwartz et al. that the immune response in the experimental animal can be abrogated by a sufficient dosage of the antimetabolite 6 mercaptopurine or other related materials (19). Appropriate manipulation of the drug results in the elimination of the γ G response to antigen while the γ M response continues unabated. This may be appropriately termed a "block" in γ G production and a simultaneous "locking" of immunoglobulin formation in the γ M position. Curiously however, the passive introduction of a small quantity of specific γ G (derived from the antibody produced by the same antigen in another animal) results in a rapid fall in γ M and then a rise in γ G. Thus this experimentally induced "lock" and "block" are at least, in this instance, reversible and

apparently the end result of a disturbance in a normal "feedback" mechanism. This seems to be dependent upon the presence of a certain amount of specific γ S when this is supplied either actively or passively the immune response proceeds in its usual fashion.

An increase in specific macroglobulin is a normal development in the humoral antibody response more specifically in its first phase. Some infections chiefly of the viral type result in a striking increase in macroglobulin as in primary atypical pneumonia in which cold hemagglutinins are common. This increase is either due to a specific and marked reaction to the antigen involved or possibly to the development of a specific group of cells concerned with γ M production. In infectious mononucleosis a variety of macroglobulins may develop: heterophile agglutinin, "syphilitic" antibody, cryoglobulin and cold hemagglutinin. The extraordinary abnormal lymphocytosis of this condition, the presence of deep blue staining lymphocytes showing marked RNA production and having many of the morphologic characteristics of immunoblasts suggest the possible development of groups of abnormal immunocytes in response to a presumed virus. In certain cases of this disease autoimmune reactions develop: a hemolytic anemia or thrombocytopenic purpura; this brings up the possibility that a reaction of normal cell antigens with abnormal immunocytes might eventuate in the autoimmune disorders found. Thus we

have postulated for various autoimmune disorders.

In certain cases of primary atypical pneumonia the concentration of cold hemagglutinins becomes unusually high. Successive attacks of this disorder may develop and this may be associated with an extraordinary rise in the titer of cold hemagglutinin to levels of 1:1,000,000 or more. If this occurs an immunohemolytic anemia may develop. Cold hemagglutinin disease, a chronic disorder, may be the culmination of repeated attacks of primary atypical pneumonia or other viral disorders which have resulted in the development of an increasingly high titer of cold hemagglutinin (CH) associated with a mild hemolytic anemia and a variably positive Coombs test. That the antibody is directed against the I red cell antigen (a self antigen) is strongly indicative of its autoimmune nature. The antibody nature of CH is clear that it is also a macroglobulin is also evident. However even with titers of CH as high as 1:10 million there may be no indication of an increase in immunoglobulin on the electrophoretic strip. On the other hand the continued study of such cases over a period of years may eventually demonstrate one or both of the following: a "spike" in the γ G area and either a blood or bone marrow lymphocytosis (or both). In other words what is at first "cold hemagglutinin disease" may in some cases eventuate in characteristic "macroglobulinemia" or in chronic lymphocytic leukemia or in both these disorders.

The development of macroglobuli

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It has been demonstrated by Schwartz et al. that the immune response in the experimental animal can be abrogated by a sufficient dosage of the antimetabolite, 6 mercaptopurine or other related materials (19). Appropriate manipulation of the drug results in the elimination of the γ G response to antigen while the γ M response continues unabated. This may be appropriately termed a "block" in γ G production and a simultaneous "locking" of immunoglobulin formation in the γ M position. Curiously, however, the passive introduction of a small quantity of specific γ G (derived from the antibody produced by the same antigen in another animal) results in a rapid fall in γ M and then a rise in γ G. Thus this experimentally induced "lock" and "block" are at least, in this instance, reversible and

apparently the end result of a disturbance in a normal "feedback" mechanism. This seems to be dependent upon the presence of a certain amount of specific γ S when this is supplied either actively or passively, the immune response proceeds in its usual fashion.

An increase in specific macroglobulin is a normal development in the humoral antibody response more specifically in its first phase. Some infections chiefly of the viral type result in a striking increase in macroglobulin as in primary atypical pneumonia in which cold hemagglutinins are common. This increase is either due to a specific and marked reaction to the antigen involved or possibly to the development of a specific group of cells concerned with γ M production. In infectious mononucleosis a variety of macroglobulins may develop: heterophile agglutinin "syphilitic" antibody cryoglobulin and cold hemagglutinin. The extraordinary abnormal lymphocytosis of this condition, the presence of deep blue staining lymphocytes showing marked RNA production and having many of the morphologic characteristics of immunoblasts suggest the possible development of groups of abnormal immunocytes in response to a presumed virus. In certain cases of this disease autoimmune reactions develop: i.e. hemolytic anemia or thrombocytopenic purpura; this brings up the possibility that a reaction of normal cell antigens with abnormal immunocytes might eventually in the autoimmune disorders found. This we

have postulated for various autoimmune disorders.

In certain cases of primary atypical pneumonia the concentration of cold hemagglutinins becomes unusually high. Successive attacks of this disorder may develop and this may be associated with an extraordinary rise in the titer of cold hemagglutinin to levels of 1:1,000,000 or more. If this occurs an immunohemolytic anemia may develop. Cold hemagglutinin disease, a chronic disorder, may be the culmination of repeated attacks of primary atypical pneumonia, or other viral disorders which have resulted in the development of an increasingly high titer of cold hemagglutinin (CH) associated with a mild hemolytic anemia and a variably positive Coombs test. That the antibody is directed against the I red cell antigen (a self antigen) is strongly indicative of its autoimmune nature. The antibody nature of CH is clear; that it is also a macroglobulin is also evident. However, even with titers of CH as high as 1:10 million there may be no indication of an increase in immunoglobulin on the electrophoretic strip. On the other hand the continued study of such cases over a period of years may eventually demonstrate one or both of the following: a "spike" in the γ G area and either a blood or bone marrow lymphocytosis (or both). In other words, what is at first "cold hemagglutinin disease" may in some cases eventuate in characteristic "macroglobulinemia" or in chronic lymphocytic leukemia or in both these disorders.

The development of macroglobuli

nemia in the course of apparently typical cold hemagglutinin disease is of considerably theoretical interest. Here, a presumably benign immunologic disorder, in which the cold hemagglutinin appears to be an autoantibody and is responsible for the mild hemolytic anemia and the abnormal reactions to cold, develops into (or is followed by) the apparently malignant disorder known as macroglobulinemia. It seems clear that cold hemagglutinin is a macroglobulin, perhaps identical with the macroglobulin of macroglobulinemia, which is now present in sufficient concentration to elicit a "spike" in the γ G area of the electrophoretic pattern. Should these conditions of cold hemagglutinin disease and of macroglobulinemia be considered as separate diseases or are they simply different expressions of the same fundamental disorder?

My own predilections in this matter are based on both the influences derived from clinical "experiments of nature" and experimental observations both indicate a close relationship and even, at times an actual identity between certain autoimmune disturbances and certain kinds of neoplastic processes of the immunocytes that I have called immunoproliferative disorders. Thus, not only do many cases of chronic lymphocytic leukemia and lymphosarcomatosis show various features of autoimmunity during their course, but there are cases of autoimmune disease — hemolytic anemia, thyroiditis, Sjögren disease, systemic lupus — which may eventuate in various forms of lymphoproliferative

disease (20, 21). New Zealand black mice with autoimmune hemolytic anemia often develop (10 percent) a generalized "lymphomatous" disorder (22). Aleutian mink with a diffuse hypergammaglobulinemia and various immunologic disorders may develop (10 percent) the typical clinical and laboratory features of multiple myeloma with a γ globulin "spike" (23). The graft vs host disease (runt disease, homologous disease) of the experimental mouse is often followed — in the recovered animal — by a generalized neoplasia of the lymphoid tissue (24). In all these instances, an abnormal immunologic disorder eventually becomes a neoplasia of the leukemic variety, i.e., an immunoproliferative disorder.

The "immunoproliferative" disorders, as I think of them, are abnormal, usually neoplastic disturbances of the cells concerned with the immune mechanism, i.e., the immunocytes comprising lymphocytes, plasmocytes, and the reticuloendothelial cells. I have included amongst these disorders chronic lymphocytic leukemia, generalized lymphosarcomatosis, macroglobulinemia, and "heavy chain" disease, all of lymphoid origin; multiple myeloma — plasmocytic, Hodgkin's disease, certain forms of reticulosis and possibly sarcoid, of reticuloendothelial cell origin. In view of the immune potentiality of the cells concerned in these neoplastic processes, it should not be considered strange that immunologic aberrations are almost always present in these conditions. Thus autoimmune disturbances as well as various forms

of dysgamma globulinemia (hypo gamma hypergamma "spikes" of γG — rare — or of γM globulin) are common in the lymphoproliferative disorders. In multiple myeloma "monoclonal gammopathy" is the rule except in the "light chain" variety in which only Bence Jones urinary proteinuria is found. In Hodgkin's disease and sarcoïdosis anergy is almost always present.

Autoimmunity may have several pathogenetic mechanisms. In my own thinking, the Burnetian concept of the "forbidden clone" has loomed large (2, 26, 27). This is based largely on the inferences drawn from clinical data both human and animal and on the experimental data already discussed. It may be hypothesized that a "forbidden clone" of immunocytes is composed of cells of a special cell line having the capacity to react with a specific self antigen. The "forbidden clone" may therefore be thought of as an abnormal "subversive" or "foreign" group of immunocytes of identical genetic configuration which may conceivably live in tolerance with the host for some time but which may on the other hand become of clinical importance if tolerance is lost. It may either be genetically derived or transiently transmitted produced by an infection or continuously "fed" by continued infection or it may be the endresult of a non-lethal mutation. Possibly some abnormal clones may have their origin in the thymus. In any event the hypothetical clone may at first result in no apparent effects later with increasing

size an autoantibody may be demonstrated (cold hemagglutinin antithyroid antibody rheumatoid factor etc.) still later, autoimmune disease may be present — presumably because the clone has expanded sufficiently so that selfantigens reacting against it will result in harmful self or autoantibodies. Eventually in some cases expansion of the presumed clone may proceed to such a degree that what we call leukemia or "lymphoma" — i.e., a gross, generalized tumor — develops with all its various features. The disorder of macroglobulinemia may represent such a condition.

From the very first studies of macroglobulinemia as made by Waldenström (1914) the bone marrow was found to contain a preponderance of lymphocytes with plasma cells either low normal or perhaps slightly increased. Thus although the condition had many resemblances to multiple myeloma the main difference at least from the standpoint of the marrow lay in the lymphocytosis as opposed to the morphology of multiple myeloma i.e. plasmacytosis. Although the lymphocytosis in the marrow is usually striking there has apparently been a reluctance to call the cells concerned typical lymphocytes or to designate the condition present as lymphocytic leukemia even in the presence of the characteristic blood picture of that disease. Thus such names as "reticulo lymphocytosis", "mixed up" lymphocytosis, "para lymphocytes", "lymphocytes with naked nuclei", "lymphoid reticulum cells" etc. have been used for the morphologic

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picture in the marrow. Our own studies indicate that the preponderant cells present have all the morphological features of lymphocytes, that the lymphocyte morphology varies from case to case or in the same case, i.e., from small to medium to large lymphocytes with a varying number of reticulum cells and "immunoblasts." To be sure, "atypical" lymphocytes are often present, as they are in some cases of chronic lymphocytic leukemia or lymphosarcoma. Plasma cells in low or normal values have been described.

It is possible to infer that the abnormal lymphocytosis of the bone marrow is related to the macroglobulinemia, just as in multiple myeloma the plasmocytosis is related to the γ G hyperglobulinemia. This would imply that the lymphocytosis is productive of the macroglobulinemia, and again by implication, that the lymphocyte can be productive of macroglobulin. Such conclusions from this and other "experiments of nature" have often cast a bright light upon hitherto obscure metabolic abnormalities. That macroglobulins are actually produced by lymphocytes is difficult to "pull down", however, although there is much suggestive evidence for this possibility. In Waldenström's macroglobulinemia the studies of Zucker-Franklin et al using both C^{14} labeled lysine cultures of lymphoid tissues together with immunofluorescent techniques have clearly indicated that medium and large lymphocytes were synthesizing macroglobulin (28). Whether some of the lymphocytes

called "large" were actually immunoblasts could not be gleaned from this study, but Figure 2 in their paper showing an imprint of a node shows very large primitive appearing cells characteristic of the immunoblast. It would be tempting to conclude at this time that, as with γ G hyperglobulinemia of multiple myeloma and its plasmocytosis, the γ M hyperglobulinemia of the macroglobulinemia is directly related to the lymphoproliferative reaction. Since the mature lymphocyte has no visible ergastoplasm and seems otherwise ill adapted to the production of protein, it may well be that the more immature lymphocyte and especially the "de-differentiated" lymphocyte or immunoblast with its large complement of ribosomes may be productive of the macroglobulin.

The production of macroglobulin, the marked lymphocytosis often of an abnormal type, the paucity of plasmocytes and of γ G globulin in most cases bring up the question of an acquired abnormality in the normal lymphoid response to antigenic stimulation, i.e., a disorder of the immunoproliferative mechanism. It is conceivable that an "insult" — whether chemical radiation or viral could delete or otherwise modify a key enzyme involved either in the transformation of lymphocyte to immunoblasts or in the development of the plasma cell (from immunoblast? from lymphocyte?). Either or both of these possibilities could result in an excessive or uninhibited production of 19S globulin as compared with 7S production. This might be thought of as analogous with the

"locking" of immunoglobulin production in 19S formation by the use of an immunosuppressive drug with a simultaneous block of 7S globulin synthesis as already noted (8). It is furthermore conceivable that such an acquired disturbance of the "feedback" shutoff mechanism could become a permanent one and thus react immunologically in a self-perpetuating fashion with identical antigens. Such an abnormality could eventually become clonal and finally generalized thus resulting in the various laboratory and clinical features of macroglobulinemia. Is this disorder then to be considered a neoplastic process, or a disorder of protein metabolism i.e. a dysgammaglobulinemia? Actually it is both. A neoplasm may be defined as a self-perpetuating proliferation of a new "race" or clone of cells. This clone composed of cells having the identical genetic background may arise in various ways (somatic mutation, viral modification, enzyme deletion etc.). In order to flourish the clone must possess a certain ecological advantage thus allowing it to proliferate at the expense of normal cell lines. A neoplastic clone of immunocytes being derived from immunologically competent cells could conceivably be subject to antigenic stimulation and thus be productive of immunoglobulins. In this instance of the γ M variety initially the macroglobulinemia might be occult i.e. found only by electrophoretic or other investigation of the serum; later it might be present in sufficient concentration to result in cold hemagglutinin disease

finally, with continued expansion of the presumed clone, it could result in the disease macroglobulinemia.

To return to the question in our introductory paragraph it would seem that in macroglobulinemia we are dealing with an aberrant, self-perpetuating disorder of immunoglobulin production with both immunologic and leukemic relevance. That an abnormality of protein production is present is self-evident. That this is a neoplastic process based on a possible mutation in which a key enzyme has been lost thus leading to the continued production of macroglobulin is of course speculative but worthwhile pursuing experimentally.

Jan Waldenström's many contributions to the "immunoproliferative disorders" even though he may wince at the term — (not that he has not concocted some himself!) — have opened our eyes to the wonders of the "gammopathies" of which he is without question The Compleat Master!

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L'heterogenete des Proteines Myelomateuses

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La microheterogenete est un caractere fondamental des immunoglobulines normales chez l'homme comme chez les diverses especes animales etudiees. Toutes les familles d'immunoglobulines actuellement individualisees apparaissent formees d'un spectre continu de molecules aux structures ioniques variant graduellement, certaines presentent des degres divers de polymérisation sans cependant que soit realise un polymorphisme analogue a celui presente par d'autres proteines seriques (hapoglobines, transferrines) ou par les isoenzymes.

Suivant la conception de Burnet (6) la production des immunoglobulines est le fait de multiples clones cellulaires dont chacun synthetise un type moleculaire strictement defini tant par des capacites fonctionnelles d'anticorps que par sa structure. Lors de l'expression immunitaire l'organisme sollicite par les multiples structures antigeniques de l'agent pathogene repond par une reaction "polyclonale" et produit une gamme etendue de molecules immunoglobuliniques d'un aspect en bande large de l'hyperglo-

bulinémie plasmatique. A l'opposé dans certaines proliferations des cellules immunologiquement competentes sont produites en abondance en l'absence d'un stimulus antigenique connu des immunoglobulines parentes de celles du sujet sain mais d'homogenete anormale cependant que se manifeste en general un deficit de la production des constituants normaux. Il s'agit alors selon la conception de Waldenström (39-40) d'une "gammapathie monoclonale" due à la proliferation de cellules issues vraisemblablement d'un clone unique mais rien ne permet de decider si ces molecules d'aspect pathologique ont une structure anormale ou si elles sont representees à l'etat de traces dans le serum du sujet sain.

Mais malgré leur homogenete physique et structurale frappante les globulines myelomateuses et les macroglobulines de Waldenström ne sont pas strictement monomorphes. Homogènes quant à leurs caracteres antigeniques et genetiques elles presentent très frequemment une heterogenete physique systematisee veritable poly-

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génique et une composition peptidique semblables (2)

Lorsque la PBJ appartient au groupe II de Korngold ("L") elle existe la plupart du temps sinon constamment (Gally et Edelman 19) sous forme de dimère stable du à la formation d'un pont disulfure. Lorsque par contre la PBJ est du type I de Korngold ("K") la polymérisation est essentiellement de caractère non covalent il existe un équilibre monomère — dimère qu'on objective la dépendance de la constante de sédimentation vis à vis de la concentration protéique

b) — *L Hétérogénéité polymorphique*
 L'hétérogénéité montrée en gel d'amidon par certaines PBJ peut correspondre la présence de constituants de constante de sédimentation identique et d'antigénicité commune mais qui montrent de légères différences de comportement thermique et se différencient à l'étude des cartes peptidiques par des divergences portant sur un ou deux peptides. Après réduction et alkylation l'électrophorèse en gel d'amidon en tampon urée formate montre ici l'existence de plusieurs bandes (Van Eijk et col 14)

Cette hétérogénéité polymorphique que l'on va retrouver dans le cas des immunoglobulines γG myélomateuses peut ici se superposer à l'hétérogénéité polymérique

L hétérogénéité des globulines myélomateuses γG

C'est l'électrophorèse en gel d'amidon qui a permis d'objectiver cette hétérogénéité aussi bien chez l'homme

(Owen, Got et Silberman 28, Engle et Woods 15 Flynn et Slow 18 Scheurien 38) que chez la souris porteuse d'un plasmocytome transplantable (Askonas 1). Il s'agit d'un aspect en multiples bandes régulièrement disposées (Fig 1 b) dont à pH 8,6 l'importance quantitative décroît avec la mobilité anodique. Il ne s'agit pas ici d'un équilibre de formes métastables séparées par chromatographie sur cellulose échangeuse d'ions (Fahey 16) ou par élution à partir du gel d'amidon ces fractions retrouvent leur mobilité et leur rapport quantitatif après nouvelle migration électrophorétique en gel d'amidon (Richard 36). Elles ont même comportement en gel filtration, même constante de sédimentation (Putnam et Udén 31). Elles ont une antigénicité commune (Fahey 16 Scheurien 38).

Il apparaît donc s'agir d'une hétérogénéité purement électrique dont l'aspect discontinu en bandes s'oppose au spectre continu des γG physiologiques elles aussi homogènes au point de vue dimensionnel. L'étude des subunités de la molécule permet d'en préciser la nature.

Après réduction et alkylation d'une protéine myélomateuse γG l'électrophorèse en gel d'amidon en milieu contenant de l'urée montre que le fragment "L" (chaîne légère) diffère profondément dans son aspect de celui qui provient de gammaglobulines normales cependant que le fragment H (chaîne lourde) diffère peu de son homologue normal.

En tampon glycine urée ou tris citrique urée l'électrophorèse en gel d'amidon

morphisme qui s'oppose à la microheterogenéité de leurs homologues physiologiques. Ce caractère doit sembler il, être rattaché selon les cas à l'heterogenéité des structures primaires (γG globulines myelomateuses), ou à l'existence de la polymérisation d'une unité structurale de base (γA et γM globulines), il peut participer de l'un et l'autre de ces mécanismes et sa nature reste encore parfois incomplètement précisée.

Il convient, de dissocier du problème de l'heterogenéité des protéines myelomateuses celui que pose la coexistence, chez le même malade de plusieurs globulines anormales. S'il est banal de trouver dans les urines, à côté de la protéine de Bence-Jones une protéine myelomateuse d'origine sérique, il est par contre rare de voir dans le sérum une protéine de Bence-Jones d'abondance suffisante pour donner un pic anormal sur l'électrophoregramme bien distinct de celui de la globuline myelomateuse γG ou γA (5 cas sur 480 dans notre statistique personnelle). — Il peut par ailleurs arriver qu'à la prolifération d'une globuline myelomateuse s'associe non un déficit de la production des autres immunoglobulines, mais une hyperglobulinémie réactionnelle, de type polyclonal dont l'importance peut être suffisante pour faire discuter une double anomalie.

L'heterogenéité des protéines de Bence-Jones (PBJ)

L'heterogenéité physique des PBJ a été mise en évidence bien avant que

fut connue leur signification nosologique, par l'ultracentrifugation (Putnam 32) l'électrophorèse en veine liquide (Putnam et Stelos 33), la précipitation par les sels neutres (L'électrophorèse en gel d'amidon (Engle et Woods 13 —, Flynn et Stow 18 —, Creysset et Fine 10), la chromatographie sur cellulose échangeuse d'ions (Lynn Phelps et Putnam 26) la gel filtration (Bernier et Putnam 5) ont permis d'objectiver plus nettement l'existence de plusieurs fractions dans les PBJ produites chez un même sujet (fig 1 a). Si on met à part les fausses hétérogénéités, liées à la présence d'autres protéines urinaires ou à la dégradation accidentelle de la PBJ, il semble à la suite des recherches de Bernier et Putnam (5) de Van Eijk, Monfoort et Westenbrink (14) que l'on puisse distinguer pour la PBJ deux types d'hétérogénéités.

1) — *L'heterogenéité polymérique*

La PBJ est formée (Edelman et Gally 13) de chaînes polypeptidiques légères "L" identiques à celles obtenues à partir des immunoglobulines myelomateuses sériques lors du clivage par réduction. Son homologue normal est constitué par les gamma micromoléculaires physiologiques.

Elle peut exister sous forme de monomère de la chaîne L (P.M. 22 000 environ) plus souvent sous forme de dimère (P.M. 44 000), parfois sous forme de tétramère. Isolés par chromatographie sur cellulose échangeuse d'ions ou gel filtration (5) séparés au cours de la migration en gel d'amidon ces composants ont une structure anti-

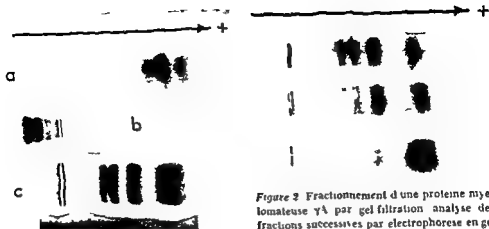


Figure 1 Electrophorese en gel d'amidon en tampon borate pH 8.6 de diverses proteines myelomateuses

- a) Proteine de Bence Jones
- b) Proteine myelomateuse γ G
- c) Proteine myelomateuse γ A (le diagramme d'immunoelectrophorese en gel d'amidon est figure 2)

constituants (Fig 1 c) dont la distribution systematisee evoque l'image des haptoglobines 2-2 et 2-1 (Creyssel Manuel Richard et Fine 11) dans d'autres cas l'heterogeneite est moins nette limitee a l'existence de deux fractions elle manque enfin parfois

Une correlation peut etre etablie entre l'heterogeneite dimensionnelle et celle que montre la migration en gel d'amidon grace a l'isolement par chromatographie sur cellulose échange d'ions (Fahey 16 Creyssel Richard Manuel et Fine 12) ou par gel filtration (Richard 3) de diverses fractions des globulines myelomateuses γ A ($F_{10} 2$). Les constituants dont la migration est la plus retardee en gel d'amidon correspondent a ceux

Figure 2 Fractionnement d'une proteine myelomateuse γ A par gel filtration analyse des fractions successives par electrophorese en gel d'amidon

qui sont le plus precocement elues lors de la gel filtration et aux constituants lourds definis par l'ultracentrifugation (9 11 13 S) a l'inverse la fraction qui contient le constituant leger 6.6 S correspond au constituant dont la mobilite anodique est la plus grande dans le gel d'amidon

— On peut donc penser que l'heterogeneite electrophoretique des proteines myelomateuses γ A est essentiellement de type *polymerique* comparable a celle presente par certaines PBJ ou par les macroglobulines. Le gel d'amidon par son pouvoir de filtration ne ferait qu'objectiver l'inhomogeneite dimensionnelle. Fahey (16) Levin et col (20) ont montre que sous l'action du microperoxyde les constituants γ A de S_{20} eleve sont dissocies et disparaissent a peu pres completement au profit du constituant 6.6 S. Et nous avons pu noter que les fractions "lourdes" separees par gel filtration semblaient apres stockage se transformer partiellement en fractions legeres

don résoud en effet les chaînes γ L normales en 10 à 12 constituants régulièrement disposés dans le gel (Cohen et Porter 8, Poulik 30). Les chaînes L provenant de globulines myélomateuses γ G, dans les mêmes conditions ne donnent par contre qu'un nombre restreint de bandes (6 au maximum pour Cohen 8), bandes de mobilité identique à celles des précédentes et ceci qu'il s'agisse de chaînes de type Kappa ou lambda (Cohen et Gordon 7).

La chaîne L¹ myélomateuse n'est d'ailleurs peut-être pas seule en cause dans la genèse de l'hétérogénéité des globulines γ G. Migita et Putnam (27) ont montré le caractère hétérogène du fragment F₁ purinique provenant des gammaglobulines normales et ses variations d'aspect lorsqu'il provient de globulines de myélome ce qui ont confirmé plus récemment Poulik et Shuster (31). Askonas et Fihely (2) ont étudié les différences d'aspect des hydrolysats peptidiques préparés à partir de fractions séparées chromatographiquement dans une globuline myélomateuse γ G de souris.

Il semble donc qu'ici comme dans le cas des P₁H₁J — et contrairement à certaines constatations initiales — puissent exister en divers secteurs de la protéine myélomateuse des facteurs d'hétérogénéité électrique.

L'hétérogénéité des protéines myélomateuses γ A

— Il existe une *hétérogénéité dimensionnelle certaine* des globulines myélomateuses γ A, démontrée par les don-

nées de l'ultracentrifugation. Déjà notée par Kechlik (23), elle a été étudiée par nombre d'auteurs (Putnam et Udén 34, Rundles, Cooper et Willet 37, Petermann, Hamilton et Koenig 29, Creysse, Cille, Coulon et Morel 24, Laurell 24). À côté du composant 6,6 S ces protéines comportent des fractions de constante de sédimentation plus élevée, 8, 11, 13 et éventuellement 15 S, dans certains cas la prédominance des fragments lourds peut justifier le terme de *macroglobulinémie atypique* employé par Jahnke et Scholtz (22). Les préparations purifiées de γ A globulines physiologiques contiennent d'ailleurs (Heremans 21) de petites quantités de ces constituants lourds.

La périodicité que présentent les valeurs de S_{20} de ces constituants évoque l'existence d'une polymérisation à partir d'une molécule monomère de poids moléculaire 150 000 environ comme l'ont confirmé récemment Levin, Ritzmann, Secuwen et Nanninga (25).

Les protéines myélomateuses γ A montrent avec une grande fréquence une *inhomogénéité électrophorétique* l'image en "bande étroite" est ici moins nette que dans le cas des myéloblomes γ G et parfois même la séparation en plusieurs pics lorsqu'on examine ces protéines par électrophorèse en veine liquide ou sur support. L'électrophorèse en gel d'amidon permet une résolution plus poussée dans un grand nombre de cas et ceci surtout lorsque la mobilité de la protéine anormale est élevée en veine liquide la migration à travers le gel la fractionne en un certain nombre de

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Mais il existe certains faits cependant qui concordent mal avec la conception d'un simple équilibre de polymérisation

— L'importance quantitative des divers composants de la globuline myélomateuse γA varie d'un cas à l'autre, qu'on l'apprecie par les données de la sédimentation ou par celles de l'électrophorèse en gel d'amidon, il existe un *polymorphisme* de l'hétérogénéité, que cette dernière technique met particulièrement bien en évidence, Ballicux (4), Fine, Boffa et Creysse (17) et qui a fait évoquer la possibilité d'une régulation génétique

— L'action des thiols présente des modalités quant à ses résultats utilisant le thioglycolate de sodium nous avons observé, par électrophorèse en gel d'amidon que les protéines myélomateuses γA d'hétérogénéité accentuée et systématisée n'étaient que partiellement transformées, deux constituants nettement distincts restent visibles dans le gel. Lorsque l'hétérogénéité est de caractère moins régulier, on note la réduction à un seul constituant. Auscher et Guinand (3) ont pu par ultracentrifugation préparative séparer à partir d'une globuline myélomateuse γA cryo-precipitable une fraction 6.75 S des constituants plus lourds. L'action de la cystéine fait disparaître les composants 11 et 13 S au profit du composant 6.75 S mais le composant 9 S ne semble pas modifier

— Des différences dans les cartes peptidiques des fractions légères et lourdes provenant de mêmes globuli-

nes myélomateuses γA ont été notées par Ballicux (4)

Il existe d'ailleurs, ici comme dans le cas des protéines myélomateuses γG une *hétérogénéité des sub-unités* produites par réduction ou par hydrolyse enzymatique. L'hétérogénéité des chaînes L (Poulik) (30) et des fragments tétrapéptidiques (Poulik et Shuster) (31) présente une image différente de celle trouvée pour les globulines γA normales

Ces faits permettent de penser que l'hétérogénéité des globulines myélomateuses γA n'est pas exclusivement liée à un équilibre de polymérisation. La tendance à la polymérisation pourrait varier d'un type moléculaire γA à l'autre (Heremans) (20) et l'on peut supposer que chez un même sujet le polymorphisme structural de l'unité moléculaire monomère puisse entraîner l'apparition de plusieurs types de polymères

Il faut enfin tenir compte du fait que l'hétérogénéité peut être liée à la formation de complexes entre la globuline myélomateuse et l'albumine (Heremans) (20) (Ballicux) (4) (Waldenström) (40)

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Table 1 310 cases of myelomatosis arranged after age sex, electrophoretic pattern and after the type of cellular morphology

Age years		13-30		31-50		51-70		71-80		81-84		Type of cellular morphology
Sex		male	female	male	female	male	female	male	female	male	female	
Electrophoretic pattern	α increase	—	—	2	2	—	1	—	—	—	—	I Mature
	β increase	—	1	4	6	5	9	5	7	1	—	
	γ increase	8	6	23	12	21	29	17	13	6	3	
	No changes	1	1	1	2	—	—	2	1	—	—	
Electrophoretic pattern	α increase	1	—	—	1	2	—	1	1	—	—	II Immature
	β increase	6	2	11	9	21	13	7	10	2	2	
	γ increase	2	1	5	1	8	6	8	3	1	—	
	No changes	—	—	1	—	2	—	—	—	—	—	
Total per cent		9		26		38		22		5		

form immature plasma cells dominate the picture and the bone marrow reticulum is strongly hyperplastic. Syncytial formations are numerous. In the both main types typical "myeloma cells" are found characterized of great often single nucleoli. Inclusion bodies (Russell bodies) azur rods and the variable blueish red colouring of the protoplasm are typical findings.

In the electrophoretic pattern pathological values have been accepted only if they reach outside of the normal variations (total amount).

Other methods of examination do not demand comment or detailed description.

The material is collected in Table 1—1.

1. The material arranged after age sex electrophoretic pattern and after the type of the cellular morphology (Table 1).

The myelomatosis is a typical disease of 16% and 64 per cent of the cases are

to be found at the age of 51—70 years of age. The mean age was 64 years by the first examination. Males are more often affected than females (54 per cent). The youngest man is 41 years of age and the youngest woman was 33 years of age. The oldest case was a man 84 years of age. The distribution of age and sex does not seem to be correlated neither to the cellular morphology of the bone marrow nor to the changes of the electrophoretic pattern.

The middle life span of 156 cases (information of the missing 154 cases has not been obtained) runs up to 3 1/2 years after the diagnosis was made. There is no difference between the sexes. The changes of the electrophoretic pattern have no influence on the life span. On the contrary the life span of immature myelomatosis is considerably shorter than that of mature myelomatosis and 72 per cent of the immature cases resulted in death within 2 years.

Myelomatosis

A clinical review of 310 cases

By NILS G. NORDLONSON

During the years 1932—1963, a material from more than 650 cases of myelomatosis has been collected. The exact diagnosis has been made by sterneal puncture and all cases have shown a specific and pathognomonic picture in the puncture specimen. On the basis of this exact morphological diagnosis the patients have been carefully examined in all respects. An invaluable help has been given me by many medical clinics in Sweden. 310 cases have been examined and they give a basis for a systematic elaboration. This material seems to be one of the greatest ones, which has been collected on one hand.

Principles of work. The division of the material has been made A) after the degree of the maturity of the myelomatosis — viz mature or immature plasma cell picture in the bone marrow and B) after the electrophoretic pattern — viz cases with increase of resp. alpha₂ beta₂ and gamma globulin in the blood serum and cases without disturbance in this pattern.

In the clinical picture the following

different symptoms have been recorded: I) the distribution of sex and age more over the life span, II) the proteinemia and the occurrence of protein in the urine — non specific or specific (Bence Jones' type), III) the different types of changes in the skeleton, IV) the changes in the peripheral blood and V) the alterations of the sedimentation velocity and the disturbance of the function of the kidneys estimated by the increase of the non protein nitrogen.

These five different types of clinical symptoms have been correlated to the changes of the bone marrow morphology and the electrophoretic pattern. In general the symptoms of myelomatosis are well known and in this respect the material gives no new aspects. But correlated to another in the manner above mentioned interesting features can be elucidated.

The mature myelomatosis is characterized of mature plasma cells and the engagement of the other reticular cells is less pronounced. Syncytia are seldom to be found. In the immature

beta type (5 g per cent) The highest value is found in a mature myelomatosis of the gamma type (14.5 g per cent)

In 87 per cent protein occurs in any form in the urine In 65 per cent albumine and in 22 per cent Bence Jones protein was found

In mature myelomatosis albuminuria is a common finding (72 per cent) Protein of the Bence Jones type is more frequent among immature myelomatosis There is no difference in the frequency of this protein in myelomatosis of the beta and of the gamma type also in alpha myelomatosis and cases that have no changes

Table II C The frequency of normal and abnormal protein in the urine in 310 cases of myelomatosis arranged after the electrophoretic pattern and the type of the cellular morphology

	Protein pos in urine	Bence Jones protein pos in urine	Type of cellular morphology
Electrophoretic pattern	α increase	5	I Mature
	β increase	24	
	γ increase	82	
	No changes	8	
Electrophoretic pattern	α increase	6	II Immature
	β increase	57	
	γ increase	20	
	No changes	2	

Table II D 310 cases of myelomatosis with normal and abnormal protein in the urine occurring in the various types of the disease (electrophoretic pattern and cellular morphology) Figures in parentheses = cases without protein in the urine

Protein pos in the urine					Type of cellular morphology
Electrophoretic pattern	α 4-6 g	7.0-7.9	8.0-8.9	10-14.5	
α increase	1	1	2	1	I Mature
β increase	5 (2)	4 (2)	8 (10)	7	
γ increase	4 (5)	7 (6)	49 (33)	22 (12)	
No changes	2 (1)	3	1	— (1)	
α increase	2	1	2	1	II Immature
β increase	6 (1)	2 (1)	34 (21)	13 (5)	
γ increase	2 (1)	2 (1)	11 (10)	2	
No changes	2	1	—	—	
Bence Jones protein pos in the urine					
α increase	1	—	1	—	I Mature
β increase	1	—	3	5	
γ increase	3	2	11	9	
No changes	—	2	—	—	
α increase	1	—	1	—	II Immature
β increase	3	2	13	2	
γ increase	1	2	2	1	
No changes	1	—	—	—	

II Changes of the amount of blood related to the cellular maturity and Bence-Jones' protein in the urine correlated to the cellular maturity and the electrophoretic pattern (Tables II—II D)

Mature myelomatosis are most common and occur in 60 per cent of the cases. Myelomatosis of the gamma-type is found in 52 per cent, those of beta- and alpha-type occur in resp. 40 and 4, and 4 per cent of the cases have no changes in the electrophoretic pat-

Table II A 310 cases of myelomatosis arranged after electrophoretic pattern and after cellular morphology

		Type of cellular morphology	
Electrophoretic pattern	α increase	5	I Mature
	β increase	38	
	γ increase	138	
	No changes	8	
Electrophoretic pattern	α increase	6	II Immature
	β increase	83	
	γ increase	29	
	No changes	3	

tern 74 per cent of mature myelomatosis belong to the gamma type and 69 per cent of the immature to the beta type. Myelomatosis of the alpha type is equally represented in the mature and the immature form. Myelomatosis without electrophoretic changes are more frequent in mature forms (73 per cent).

Increased blood protein (more than 8 g per cent) is found in 79 and hypoproteinemia (less than 7 g per cent) in 11 per cent. Immature myelomatosis have more often increased blood protein than mature forms (67 per cent). Myelomatosis of the gamma type have hyperproteinemia in 45 per cent but only 32 per cent of the beta type have increased blood protein. Decreased or normal amount of blood protein is more common in mature myelomatosis. Myelomatosis without changes of the electrophoretic pattern always has normal or a decreased amount of blood protein. The lowest value of the blood protein is however found in a case of immature myelomatosis of the

Table II B 310 cases of myelomatosis arranged after the amount of total blood protein, after the electrophoretic pattern and after the cellular morphology

Total blood protein in g per cent		5—7	7.1—7.9	8—9.9	10—14.5	Type of cellular morphology
Electrophoretic pattern	α increase	1 (6.4)	1	2	1	I Mature
	β increase	7 (min 5.6)	6	18	7	
	γ increase	11 (min 5.4)	13	52	34 (max 14.5)	
	No changes	3 (min 5.9)	3	1	1	
Electrophoretic pattern	α increase	2 (min 5.6)	1	2	1	II Immature
	β increase	7 (min 5.0)	3	25	18	
	γ increase	3 (min 5.7)	3	21	2	
	No changes	2 (min 6.2)	1	—	—	
Total		34 (11%)	31 (10%)	182 (59%)	61 (20%)	

Table IV 310 cases of myelomatosis arranged after various peripheral blood changes electrophoretic pattern and after the cellular morphology

		Anemia	Thrombocytopenia	Granulocytopenia	Plasma cells	Leukemoid picture	Coincidence with "blood diseases"			Type of cellular morphology
							Lymphatic leukemia	Myeloblastic leukemia	Perniciou anemia	
Electrophoretic pattern	α increase	3	2	3	1	1	—	—	—	I Mature
	β increase	23	21	16	3	2	1	—	—	
	γ increase	78	60	47	3	8	1	1	1	
	No changes	4	3	3	—	1	—	—	—	
Electrophoretic pattern	α increase	5	3	3	—	1	—	—	—	II Immature
	β increase	51	30	31	2	5	2	1	2	
	γ increase	20	21	11	1	2	1	—	—	
	No changes	2	—	—	—	—	—	—	—	

combined bone changes are more frequent in myelomatosis of the gamma type. Cases lacking any skeletal changes are more common in myelomatosis of the gamma type. The same concerns the alpha type of the disease and cases without alterations in the electrophoretic pattern.

IV Various changes of the peripheral blood correlated to the different types of myelomatosis (the maturity of the cellular morphology and the changes of the electrophoretic pattern) Table IV

Changes of the blood in myelomatosis are usually combined. Anemia and diminished platelet count occur in 60 per cent. Granulocytopenia is found in 37 per cent. In only 5 per cent of the cases plasma cells are found. Leukemoid blood picture occurs in 7 per cent.

Immature myelomatosis show more

changes in the peripheral blood than mature forms. Findings of plasma cells and leukemoid blood pictures are more frequent in the mature form of the disease. Mature myelomatosis of the gamma type shows a high frequency of blood changes as well as immature myelomatosis of the beta type. In cases of mature myelomatosis without changes in the electrophoretic pattern alterations in the peripheral blood are common.

The coincidence of "blood diseases" and myelomatosis is unusual and this combination is specially seen in immature myelomatosis of the beta type.

V Changes of the sedimentation velocity and the non protein nitrogen arranged after the cellular maturity and the type of the electrophoretic pattern Table V

Low values of the sedimentation velocity (below 30 mm in one hour) are

Table III 310 cases of myelomatosis arranged according to different bone changes occurring in the various types of the disease (electrophoretic pattern and cellular morphology)

Art of bone changes		Osteoporosis only	Gun shots only	Osteoporosis and Gun shots	Mixed changes	No skeletal changes	Type of cellular morphology
Electrophoretic pattern	α increase	—	1	2	2	—	I Mature
	β increase	9	5	13	7	4	
	γ increase	8	40	34	39	17	
	No changes	—	2	2	4	—	
Electrophoretic pattern	α increase	2	—	3	1	—	II Immature
	β increase	23	3	29	26	2	
	γ increase	1	8	10	10	—	
	No changes	—	—	2	1	—	
Total		43	59	99	90	23	

in the electrophoretic pattern. Proteinuria of any form is more common in gamma myelomatosis of the mature type but more frequent in beta-myelomatosis of the immature form.

There is no difference in the frequency of absence of proteinuria correlated to myelomatosis of various types of the electrophoretic pattern and the degree of the maturity as well as the amount of the total blood protein.

Albuminuria is more common with increased total blood protein but is the opposite with decreased blood protein, the Bence Jones protein being more common.

Albuminuria occurs in 91 per cent of myelomatosis of the beta- and the gamma types. All cases of the alpha type of the disease have albuminuria. In cases of myelomatosis missing changes in the electrophoretic pattern albuminuria is found in 73 per cent.

III Various bone changes (seen in X ray) in myelomatosis arranged according to the different types of the disease (the maturity of the cellular morphology and the changes of the electrophoretic pattern) Table III

In 94 per cent of the whole material changes in the skeleton were found.

Combined changes were most common (63 per cent) pure osteoporosis was found in 14 per cent and "gun shots" only in 17 per cent. Pure osteoporosis is frequent in immature myelomatosis. Mature types of the disease more often show "gun shots" only. Combined changes are more frequent in mature myelomatosis. Lack of any bone changes is more common in the mature form.

Myelomatosis of the gamma type has more frequent skeleton changes than the beta type. Pure osteoporosis is more common in myelomatosis of the beta type. "Gun shots" as well as

ritz) Perhaps further studies of the morphology of the plasma cell will discover other pathological forms. New details of the electrophoretic pattern will fully elucidate this interesting question.

Myelomatosis is a systemic disease (reticulosis) specially in its immature form which occur in 10 per cent of all cases. There is a widespread affection of the primitive reticular cell of the whole body. The development of a local plasmocytoma in a myelomatosis or a plasma cell leukemia make this conception true. There can hardly be a metastatic process. The myeloma cell has all signs of malignity.

The myelomatosis is a disease of age and there are no cases younger than 33 years of age. The middle life span is about $3\frac{1}{2}$ years but it is definitely shorter in immature myelomatosis (up to 2 years).

Mature myelomatosis is most common as well as the gamma type of the disease. Among the mature forms the gamma type dominates but the beta type is more usual in the immature myelomatosis.

Hyperproteinemia is frequent in mature myelomatosis and in its gamma type. Hyperproteinemia is more common in immature forms of the disease.

Albuminuria occurs principally in cases with hyperproteinemia but the Bence Jones protein is more often found in cases with low blood protein.

Skeleton changes are very frequent (91 per cent). The changes are usually combined. Cases lacking bone

changes are seen mainly in the gamma type of the disease. Pure osteoporosis is frequent in the immature forms but "gun shots" only are more often found in mature myelomatosis.

Changes in the peripheral blood are usual and they are generally combined. In immature forms of the disease they are most common. Anemia and diminished platelet count are found in 60 per cent. Findings of plasma cells are rare (3 per cent) and they occur specially in mature myelomatosis. Only one case of plasmacell leukemia has been observed but a fully clinical examination was not obtained. Leukemoid peripheral blood pictures occur mainly in mature myelomatosis. The coincidence of "blood diseases" and myelomatosis is rare and is seen in immature forms of the beta type.

The sedimentation velocity is always high and only 3 per cent of the cases showed a rate below 30 mm in one hour.

Severe damage of the function of the kidneys was found in only 4 per cent.

Myelomatosis is an interesting and fascinating disease. This material shows its manifold symptomatology. With all symptoms present the diagnosis is easy but essential symptoms can be lacking. Several symptoms can be correlated to the maintypes of the myelomatosis. Sometimes is this correlation confusing and unexpected. For instance in immature myelomatosis *a priori* more severe clinical picture ought to be expected. In cases with

Table V 310 cases of myelomatosis arranged after sedimentation velocity, non protein nitrogen, electrophoretic pattern and after the type of the cellular morphology

		Sedimentation velocity (mm/l hour)				Non protein nitrogen (mg per cent)				Type of cellular morphology
		-30	31 -50	51 -100	100-175	-40	41 -50	51 -100	100 -300	
Electro phoretic pattern	α increase	—	—	2	3	2	2	—	1	I Mature
	β increase	—	—	10	28	19	14	5	—	
	γ increase	3	4	43	88 (173)	69	33	31	5 (225)	
	No changes	1	—	4	3	3	1	2	2	
Electro phoretic pattern	α increase	—	—	4	2	1	—	3	2	II Immature
	β increase	4	6	20	53	32	28	21	■	
	γ increase	1 (16)	—	2	26 (175)	15	9	3	■ (300)	
	No changes	1	—	1	—	1	—	2	—	

rare and found in 3 per cent. High values (above 100 mm in one hour) occur in 80 per cent.

In general, there is no correlation between the alterations of the sedimentation velocity and the maturity of the myelomatosis or the changes of the electrophoretic pattern. However, low values are found in immature forms of the disease. The alpha-type has always high values as well as the beta type of the immature form.

Changes in the function of the kidneys estimated after the increase of the non-protein nitrogen (NPN) show that a normal function (NPN below 40 mg per cent) is found in 50 per cent. Severe damage in the function of the kidneys (NPN above 100 mg per cent) occurs in only 4 per cent. In general, there is no definite correlation between NPN and the degree of the cellular maturity or the changes of the electrophoretic pattern.

Comments and summary

Myelomatosis viz. multiple myeloma is a specific disease affecting the plasmacellular system. It is characterized by a variegated clinical picture with five different main symptoms: pathological plasma cells formation, alterations in the blood proteins including formation of Bence Jones protein, skeletal changes, changes of the peripheral blood and damage of the function of the kidneys. In this material all these five symptoms occur in 30 per cent.

In regard to the principles of work, the material does not give any information of cases without positive changes in the sternal puncture but with other clinical symptoms of a myelomatosis.

There seems to be a relationship between myelomatosis, makro globulinemia and "paraproteinemia" (Waldenström). But the distinction of these three entities has been criticized (Und

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Alkeran® (Melfalan) in the Treatment of Myelomatosis

By AA DRIVSHOLM MD and AA VIDEBÆK MD

A large number of drugs have been tried in the treatment of myelomatosis but with meagre results (cf review by Drivsholm 1965 (6)). L-phenylalanine nitrogen mustard (Alkeran® Melfalan) is among the most recent and more promising drugs. This agent was synthesized by Bergel and Stock (1) in 1953 and in its racemic form (sarcosine) it was applied to the treatment of myelomatosis for the first time by Blokhin, Larionov, Perevodchikova (chobotireva) and Merkulova (4) in 1958.

This is a report on 4 years experience of Alkeran in the treatment of myelomatosis. A preliminary report on part of the material has previously been published by Videbæk (17).

Material and Method

Material

The study comprises all symptomatic cases admitted to the two above mentioned medical departments during the period 12/1961—31/1965 a total of 50 patients from the final

analysis the following patients were excluded: 1) 10 patients followed for less than 3 months 2) 8 patients who could not be followed regularly in the named departments 3) 1 patient who had cancer of the lung besides myelomatosis and 4) 1 patient who had been treated also with cyclophosphamide during the observation period. The remaining 35 patients were followed by one of us throughout the treatment period. By the end of the observation period 19 of these patients had died.

The diagnostic criteria were 1) Myeloma cells in a bone marrow specimen and 2) either an M component in the serum (immunoelectrophoresis) or typical skeletal lesions (X ray).

The distribution of the material by sex, age (at the time of diagnosis) and type of M component is given in Table 1.

Principle of Treatment

Alkeran was administered either as serial treatment or as permanent treatment. In the former used during the early part of the study period the dosage was 10 mg daily for a maximum of 6 days and intervals of 3 weeks were allowed to elapse between each

divergences in the electrophoretic pattern from the usual ones viz alpha-myelomatosis and cases with normal electrophoresis, the clinical picture ought to be more penetrated

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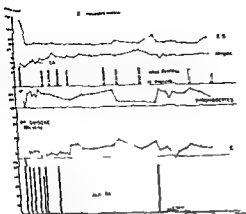


Fig 1 Biochemical changes during treatment with Alkeran® for 44 months in a case of γ A myelomatosis initially with hypercalcaemia (CA)

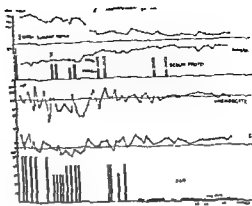


Fig 2 Biochemical changes during treatment with Alkeran® for 48 months in a case of γ C myelomatosis

Table 1 Material

30 patients	26 ♂						
	29 ♀						
Average age	62.0 ± 0.3 years (37–81 years)						
+ M component in the serum	53 pts						
	<table> <tr> <td>γC</td><td>39 pts</td></tr> <tr> <td>γA</td><td>13 pts</td></tr> <tr> <td>Bence Jones</td><td>1 pt</td></tr> </table>	γ C	39 pts	γ A	13 pts	Bence Jones	1 pt
γ C	39 pts						
γ A	13 pts						
Bence Jones	1 pt						
— M component in the serum	2 pts						
	<table> <tr> <td>Hypogammaglobulinaemia</td><td></td></tr> <tr> <td>Immunoparalysis</td><td></td></tr> </table>	Hypogammaglobulinaemia		Immunoparalysis			
Hypogammaglobulinaemia							
Immunoparalysis							

Table 2 Treatment with Alkeran (55 pts)

Duration	Average 21.4 ± 13.1 months (3–48 months)
Total dose	Average 802 ± 461 mg (110–2350 mg)
Daily dose	Average 1.32 ± 0.59 mg (0.1–2.4 mg)

Table 3 Therapeutic effect

	+effect	—effect
A Alkeran	13	3
B Alkeran+local radiotherapy	9	2
C Alkeran+prednisone	14	1
D Alkeran+prednisone+local radiotherapy	11	1
	47	8

course. The permanent treatment was started on 5 mg daily for 10 days after which the maximum dose was 2.5 mg daily. The maintenance dose was in all cases adapted according to the platelet count, white cell count and haemoglobin concentration. During the early part of the treatment, these parameters were checked once weekly, later every 3 or 5 weeks.

The average total and daily doses administered to the 55 patients are listed in Table 2 which also gives the duration of treatment.

The treatment was instituted in all cases with Alkeran. Patients in severe pain were also given short courses of radiotherapy to the isolated, painful skeletal foci. If there was vital indication for prednisone therapy (hypercalcaemia with cerebral confusion or severe nephropathy with rapidly deteriorating renal function and imminent uraemia), the treatment was supplemented by prednisone.

In 42 of the 55 patients the Alkeran therapy was instituted within 6 weeks after myelomatosis had been diagnosed. Six patients had been treated with urethane prior to the Alkeran therapy but not immediately before. The remaining patients had not previously been treated with cytostatics.

Evaluation of Therapeutic Effect

The criteria of a therapeutic effect were

- 1) > 20 % decrease in the *M* protein concentration in the serum
- 2) > 20 % increase in the albumin concentration in the serum (in the

event of a decreased albumin concentration)

- 3) > 20 % decrease in the ESR
- 4) > 20 % increase in the haemoglobin concentration (without transfusion)
- 5) > 20 % decrease in an elevated serum creatinine
- 6) Decreasing proteinuria
- 7) Normalization of an elevated serum calcium

The patients' statements concerning decreased pain and a possibility of increased mobility after the institution of Alkeran therapy were recorded but never used as the only criterion of a therapeutic effect.

Results

The therapeutic effect is given in Table 3 in which the series is grouped by method of treatment. A therapeutic effect is recorded, if only one of the above mentioned 7 criteria was fulfilled. In the majority of these cases however several of the criteria were fulfilled. Out of the 8 patients in whom no effect could be traced 7 had an M component in the serum of the IgG type, while the eight had only immunoparalysis. Of the 47 patients who gave a primary response to the treatment 10 were again showing signs of increased disease activity at the end of the observation period. Two of these patients had not been treated as intensively as might have been desired because they did not cooperate properly. Out of the remaining 8 4 had been treated with Alkeran and prednisone and 4 with Alkeran only. Fig. 1 gives the

Table 5 Side effects of Alkeran therapy (25 pts)

Expected	Trombocytopenia ($< 100\,000/\mu\text{l}$)	33 pts
	Leukocytopenia ($< 2\,000/\mu\text{l}$)	31 pts
	Increased anaemia	16 pts
Unexpected	Hair loss	1 pt
	Exanthema	3 pts
	Bone pain	3 pts
	Nausea vomiting diarrhoea	6 pts

Discussion

As emphasized initially by Waldenström (19) it is difficult to evaluate the therapeutic effect in myelomatosis as the course and prognosis in the untreated patient are extremely varying. Moreover the varying course as well as the poor prognosis (18) usually necessitate individual treatment by several drugs.

The criteria used in the present study assess the therapeutic effect correspond rather accurately to those used by Waldenström (19) in his analysis. We did not include the patients' statements concerning less pain and fatigue, increased mobility and ability to work, etc. in our assessment of the therapeutic effect — even though such changes were striking — because it is so difficult to compare subjective sensations and to rule out a suggestive influence upon the patients. In many instances the analgesic effect of Alkeran was very pronounced and manifest as early as a week or two after the medication was started. Statistical calculations of the survival curve representing the treated patients were not carried out in the present study because of the fairly short follow-up period and the lack of a comparable

untreated series of patients with myelomatosis. A fall in the percentage of plasma cells in bone marrow specimens was not used as a criterion of a therapeutic effect owing to variations from one preparation to another from the same specimen and in various specimens removed within the same 24 hours (7).

The assessment of the effect of a drug is particularly difficult when other drugs are being administered at the same time. In certain cases the demand of the best possible treatment owing to the poor prognosis of the diseases made it necessary to supplement Alkeran therapy with prednisone and local radiotherapy.

However neither of these two treatments appears to entail a general improvement in the prognosis of myelomatosis (5).

The distribution of the present series by sex, age and type of M component corresponds accurately to the distribution in a larger Danish series of myelomatosis from 1964 (2).

The favourable effect of Alkeran in myelomatosis corresponds roughly on the whole to that found by others (2, 3, 4, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 20). As none of the 8 patients with

Table 4 Therapeutic effect obtained

Alteration in per cent	20-39 %	40-59 %	60-79 %	> 80 %	Total
Decrease in M protein	5	15	14	4	38 (31)
Increased in albumin	3	2	2	11	18 (34)
Fall of ESR	8	11	13	12	44 (54)
Increase in Hb	4	4	0	1	9 (38)
> 20 % decrease in elevated serum creatinine					14 (11)
Declining proteinuria					9 (37)
Normalization of serum calcium					4 (2)

course of the disease in one of the patients who primarily went into complete remission, clinically as well as biochemically. After 21½ months without any form of treatment, the patient developed a recurrence and succumbed despite repeated Alkeran medication. This patient had initially exhibited a prompt decrease in an elevated serum calcium on prednisone. Alkeran treatment. Such an effect was observed on Alkeran alone in one out of 5 cases with hypercalcaemia. The therapeutic effect of Alkeran is often of slow onset. This is illustrated in Fig. 2 which also shows a marked increase in haemoglobin on Alkeran medication.

In Table 4 an attempt is made to grade the therapeutic effect in the total series. The figures in brackets signify the number of the patients who showed alterations of the various parameters. The M component decreased by more than 40 % in 2% of the patients, and the albumin concentration often increased. The haemoglobin concentration usually rose but nevertheless showed a declining tendency in 16 cases. In 5 cases the decrease in serum creatinine was obtained on Alkeran alone, while the remaining 9 patients

were treated with Alkeran plus prednisone. In 1 patient the serum creatinine decreased from 7.5 to 2.5 mg/100 ml on Alkeran alone. In 2 of the 9 patients with decreasing proteinuria, this decrease occurred on Alkeran alone, while the other 7 patients were receiving Alkeran as well as prednisone. There was no correlation between the average daily dose of Alkeran and changes in the parameters ESR, M protein, and albumin concentration.

Healing of osteolytic skeletal lesions did not occur in any case on Alkeran.

The side effects of Alkeran are shown in Table 5. The thrombocytopenia and leukocytopenia were often pronounced and yielded but slowly and often only partially after discontinuation of Alkeran. The tendency to leukocytopenia and thrombocytopenia was pronounced in patients with impaired renal function. Two patients died of severe infection perhaps caused by an Alkeran induced leukocytopenia. Fatal hemorrhages due to severe thrombocytopenia were not observed. As already mentioned the haemoglobin concentration showed a declining tendency in 16 patients during the treatment. All the unexpected side effects were mild.

the degree of proteinuria an effect was obtained in 80 % of the patients (Table 4). Serum creatinine as well as serum calcium and the proteinuria could be appreciably reduced by Alkeran alone. In 10 cases there were signs of increased disease activity by the end of the observation period.

Side effects in the form of thrombocytopenia and leukocytopenia were often ascertained and were especially pronounced in patients with impaired renal function. Other side effects were rare and mild.

It is concluded that Alkeran is applicable with success in the treatment of myelomatosis. However it should be used only in the treatment of myelomatosis which has been definitely diagnosed and showing manifest signs of disease activity. The present study did not answer the question whether an initiated Alkeran medication therapy should be continued after a therapeutic effect has been obtained.

Acknowledgement

We are greatly indebted to Jørgen Clausen, M.D. Head of the Institute of Neurochemistry, Copenhagen for the immunoelectrophoretic studies.

Alkeran® was generously supplied by Burroughs Wellcome & Co.

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lacking effect of Alkeran had type γ_1 myelomatosis, patients having myelomatosis of this type are perhaps on the whole more responsive to Alkeran therapy than patients with M protein of the γ_0 type in the serum. This possibility was emphasized already by Moeschlin & Jubin (11). Apparently some patients are resistant to the treatment from the very outset. However, Alkeran should be continued for a long time, before a therapeutic effect can be excluded (cf Fig 2). Other patients appear to develop resistance to the treatment for some unknown reason, so that they have to be switched over to another cytostatic. As already mentioned it may be risky to discontinue Alkeran, although the patient has entered clinical and biochemical remission (cf Fig 1). This has also been emphasized by Waldenström (19).

The favourable effect of Alkeran upon an impaired renal function is beyond doubt. Patients with impaired renal function appear to be more apt than others to develop leukocytopenia and thrombocytopenia on Alkeran therapy, but if the medication is combined with prednisone Alkeran is better tolerated. Therefore it is recommended to supplement the treatment with prednisone in the presence of definitely impaired renal function. Prednisone also appears to be indicated as an adjunct in patients with severe proteinuria and in patients with severe hypercalcaemia.

The side effects of Alkeran are as already mentioned, few and harmless if overdosage is avoided. Therefore cases of myelomatosis with pancytopenia,

due exclusively to the basic disease, should also be treated with Alkeran, in small doses, the anaemia being treated, in severe cases, by transfusion of packed erythrocytes (and possibly prednisone), the leukocytopenia and thrombocytopenia with prednisone. However, the side effects may be fatal, so that accidentally diagnosed cases of myelomatosis should probably not be treated with Alkeran, until signs of disease activity appear. Patients with an M protein component in the serum, in whom the diagnosis is not definite should not be treated until the diagnosis of myelomatosis has been confirmed. If these precautions are observed, Alkeran is an excellent, relatively non-toxic cytostatic for the treatment of myelomatosis.

Summary

Fifty five patients with myelomatosis were followed for 3—48 months during treatment with Alkeran®. The average follow up period was 21.4 ± 13.1 (S.D.) months. The total dose ranged from 110 to 2350 mg, the average daily dose being 132 ± 0.59 (S.D.) mg. Patients with severe bone pain received initially radiotherapy of isolated skeletal foci. Patients having alarming hypercalcaemia with cerebral confusion and patients with severe nephropathy were treated concurrently with prednisone. Nineteen of the patients had died by the end of the observation period.

Assessed on the basis of the alterations in the serum concentration of M protein and albumin, the L/S R IIb, serum creatinine, serum calcium and

incubated for 3, 10 and 20 minutes after which 0.2 ml fibrinogen solution or 0.2 ml normal citrated plasma was added.

The presence of fibrinolytic split products were demonstrated immunochemically (12, 13).

The myeloma globulins were classified immunochemically by Bachmann and Laurell (2) and purified by Hansson et al (6).

Results (Table I, II and III)

Of the 63 patients examined 39 had myeloma globulin type γ G (γ G myeloma) 14 type γ A (γ A myeloma) 6 type lightchain protein (γ μ myeloma) 3 had \equiv abnormal globulins (γ A + γ G / γ G + γ μ γ A + γ μ myeloma) and one patient type γ D globulin (γ D myeloma) recognizable in serum on paper electrophoresis (9). Seventeen patients had bleeding symptoms (nose bleed in 6, bleeding from oral mucosa in 1, in 1 purpura and subcutaneous haematoma) at the time of blood sampling or just before and of these 9 had γ G myeloma 6 γ A 1 γ μ and one patient γ A + γ μ myeloma.

The mean values and ranges for some coagulation factors in different types of myeloma are given in table I.

Coagulation defects were about equally common in patients with and without bleeding symptoms (Table II).

The distribution of normal low and high levels of platelets, prothrombin, APTT and pathologic fibrinolytic activity did not vary with the type of myeloma (Table III). The fibrinogen values (Table I and III) were high (0.17—1.21 g per cent) in 28 of the 39 patients with γ G myeloma. Of these 28 patients the antithrombin titre was increased in 16 as well as in 3 patients

Table I. Mean values and ranges of some coagulation factors in different types of multiple myelomas

Type of myeloma	Number of patients examined	Coagulation time (8—14 min)	Bleeding time (Duke) (1—5 min)	Prothrombin (FV, FII + FVII, FVIII & FIX)	Factor V (80—100 %)	APTT (FVII, FVIII, FIX) (60—120 %)	B factor (FII) (60—160 %)	Fibrinogen activity (60—140 %)	Fibrinolytic activity (0—50) plasma (0—70) whole blood precip	Fibrinogen g/100 ml (0.25—0.51)	Number of patients with prolonged thrombin time
γ G	39	12 (7—28)	1 (1—70)	97 (21—140)	110 (90—200)	159 (24—600)	118 (48—243)	112 (61—190)	6, 107 (0—203) (0—150)	0.48 (0.23—1.21)	10
γ A	14	13 (4—24)	3 (2—6)	81 (40—108)	111 (90—193)	116 (30—210)	83 (50—130)	102 (41—133)	16 (0—115) (0—193)	0.31 (0.10—0.62)	0
γ μ	6	13 (8—16)	3 (2—6)	116 (108—122)	141 (79—206)	230 (78—324)	144 (118—200)	108 (91—121)	18 (0—40) (51—106)	0.5, 0.5, (0.28—1.15)	0
γ D	1	11	8	116	130	192	—	91	0	0.37	0
γ A + γ G	1	16	4	58	11	117	—	88	10	0.81	1
γ A + γ μ	1	10	2	91	120	163	33	127	0	0.32	0
γ G + γ A	1	21	1	160	106	83	72	170	0	0.42	0

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Coagulation studies in different types of myeloma¹

BY JAN-ERIK NILÉN and INGA MARIE NILSSON

The occasional bleeding in patients with multiple myeloma is often due to thrombocytopenia (7). But in such patients the clot retraction may be retarded, the one stage prothrombin time prolonged and the antithrombin titre increased (5, 8, 11, 22), i.e. all abnormalities which may contribute to impaired haemostasis. Some authors (5, 11, 22, 23) ascribe these coagulation defects to the myeloma globulins.

The purpose of present investigation was to ascertain whether any association exists between the coagulation pathnes particularly the antithrombin effect and different myeloma globulins.

Material and methods

Clinical material 63 patients 24 men and 39 women aged 38–86 of the department of medicine Malmö general hospital were examined. The diagnosis of myeloma was based on roentgenological skeletal changes demon-

stration of pathological proteins (M components) in the plasma and the presence of an increased number of atypical plasma cells in the bone marrow.

Methods The blood was collected with silicone technique and citrated plasma and serum were prepared as described previously (12–19). As a rule the coagulation studies included determination of the coagulation time in glass and plastic tubes, bleeding time, recalcification time of citrated plasma, prothrombin consumption, prothrombin and factor VII factor V one stage prothrombin time, APTT (if VIII) and haemophilia B factor (if IX), fibrinogen assays of fibrinolytic activity, plasminogen activity and inhibitors of plasminogen activation were performed by methods described elsewhere (16–17, 18, 19). The thrombin time was determined in the way described earlier (12) and was said to be prolonged when it exceeded the control with 10 seconds in citrated plasma samples coagulated with thrombin (Topostasin Roche 3 NIH ml). The isolated globulins were dissolved in a phosphate buffer (pH 7.0 0.05 M) in 0.5 M NaCl solution and tested for their antithrombin effect in the following system: a) 0.2 ml 0.4% per cent human fibrinogen solution (Kabi) + 0.2 ml of globulin solution (or buffer for control) + 0.2 ml of thrombin solution (3 NIH per ml); b) a mixture of 0.2 ml globulin solution (or buffer for control) and 0.2 ml of thrombin solution (3 NIH ml) was

¹ This investigation was supported by grants from the Swedish Medical Research Council and the Medical Faculty, University of Lund.

The AIT values were increased in 24 normal in 35 and low in 3 patients (Table I and II)

Discussion

It is clear from the results given above that a variety of defects may occur in the coagulation mechanism of patients with multiple myeloma. Of our 63 patients 17 had bleeding symptoms. The coagulation defects did not appear to vary in frequency or type with the presence or absence of bleeding symptoms (Table II). As expected thrombocytopenia was common. The fibrinolytic activity was often abnormally increased and the fibrinogen level was elevated in half of the patients (Table I and III). Of special interest was the high antithrombin titre in multiple myeloma (5, 11, 22, 23). It was found in 20 patients including 19 with γG myeloma and one patient with $\gamma A + \gamma M$ myeloma. In none of the patients could a low fibrinogen value explain the prolonged thrombin time. The fibrinogen value was normal in 3 of the patients and high in 17 with a high antithrombin titre. The fibrinogen level could not explain the high antithrombin titre (10) because it was normal in the other patients with myeloma and high fibrinogen level. We also determined the antithrombin titre in myocardial infarction, pneumonia and other infectious diseases with high fibrinogen values (0.45–0.95 g per cent) but none of them had a prolonged thrombin time. No correlation seems therefore to exist between high antithrombin titre and high fibrino-

gen levels in myeloma patients. Individual differences in the thrombin binding capacity of the fibrinogen molecule may exist but this was not investigated.

The absence of a demonstrable anti-coagulant effect in the first stage of coagulation excluded the possibility of a circulating anticoagulant of type antithrombin II (heparin + heparin co factor) (4).

A possibility hitherto not considered in myeloma patients is that the amount of split products arising on digestion of fibrinogen by plasmin (1) might be sufficient to inhibit the conversion of fibrinogen to fibrin. In 12 of the patients with a high antithrombin titre an abnormally high fibrinolytic activity in plasma was found and comparative immunoelectrophoresis revealed fibrinolytic split products in 4 of 8 studied. This suggests that split products may help to explain the high antithrombin titre in some myeloma patients but they cannot be responsible for the prolonged thrombin time in most of the patients.

Like other workers in this field (5, 11, 22) we thought that the abnormal globulin occurring in myeloma was responsible for the prolonged thrombin time. Isolated γG globulin (6) from 3 of our patients with prolonged thrombin time was tested in different systems with γG globulin concentration between 1.4 and 1.8 g per cent. These γG globulins were found to have no antithrombin effect. This was incompatible with the findings reported by earlier investigators (5, 11, 22, 23) who however did not work

Table II Frequency of some coagulation defects in 17 patients with and 46 without bleeding symptoms

	Prolonged coag time	Prolonged bleed time	Decreased number of platelets	Decreased prothrombin	Prolonged one stage prothrombin time	Decreased ATIII	Decreased fibrinogen	Increased fibrin lysis
With bleeding symptoms	8	4	9	6	4	3	3	1
Number of patients studied	16	17	17	17	17	17	17	17
Without bleeding symptoms	15	6	18	8	7	0	0	20
Number of patients studied	45	40	38	44	44	43	44	44

Table III Distribution of normal, low and high values of some coagulation factors in patients with γG , γA and $\gamma \mu$ myeloma

Type of myeloma globulin	Platelets	Prothrombin	Fibrinogen	Fibrinolytic activity	Split products	Antithrombin titre	ATIII
γG { Normal	17	27	10	17	8	10	23
Low	13	7	—	—	—	—	1
High	3	3	28	21	7	10	12
γA { Normal	7	8	9	8	1	14	9
Low	4	6	3	—	—	—	2
High	1	0	1	5	11	0	3
$\gamma \mu$ { Normal	3	3	3	2	0	11	1
Low	2	1	11	—	—	—	—
High	1	2	3	4	1	0	5

with γG myeloma with normal fibrinogen value (0.23—0.34 g per cent). The patient with $\gamma A + \gamma \mu$ myeloma also had a high antithrombin titre and a high fibrinogen value (0.81 g per cent). In the patients with γA myeloma the fibrinogen values ranged from 0.19 g per cent to 0.62 g per cent but in none of the 14 patients with γA or the 6 with $\gamma \mu$ myeloma was the antithrombin titre increased. Nineteen patients were studied for fibrinolytic split products. In 8 of them the serum contained such products. Serum

punctate from 5 patients without any demonstrable split products in the serum from venous blood showed pronounced fibrinolytic activity in sternal plasma on unheated fibrin plates (100—300 mm²). Fibrinolytic split products were also found in sternal serum without (all 5 patients) and with $\gamma AC \lambda$ (3 patients).

The inhibitors of plasminogen activation by urokinase or streptokinase in 34 patients studied proved normal in 14, decreased in 1 and increased in 7.

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with purified preparations, but was in accord with the findings of Perry (20) in his investigation of plasma fractions from myeloma patients.

The thrombin binding capacity of the γ G globulin may, of course, be destroyed during preparation of the pure globulin. Besides our test system may not be sensitive enough to detect the defects. Further investigation of the myeloma globulins is therefore desirable.

The antithrombin effect noted in multiple myeloma is still obscure but it may be due to a) fibrinogen degradation products b) different thrombin binding capacity of fibrinogen or c) the presence of some other protein in increased amounts in particularly γ G myeloma.

Sirridge et al (21) described 2 myeloma patients with fibrinolysis. In our myeloma material the frequency of fibrinolysis was remarkably high compared with fibrinolysis in a clinical material of coronary infarction and pneumonia with high and normal fibrinogen values (14). This difference is difficult to explain. It may be due to the involvement of the bone marrow in multiple myeloma. The presence of split products in sternal serum and/or in venous blood may depend of the degree of the activity of the myeloma as may the high fibrinogen and AHT (F VIII) (3).

Summary

In 63 patients with multiple myeloma 17 were found to have bleeding symptoms. Coagulation defects of the same

type occurred in patients with and without bleeding symptoms. Fibrinolysis was found in half of the patients and high fibrinogen values were equally common.

The thrombin time was prolonged in 20 of the patients investigated. In 19 of them the disease was classified immunochemically as γ G myeloma and in one as γ A+ γ M myeloma. The high antithrombin titre could not be ascribed to the myeloma globulin or to the high fibrinogen values noted.

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Cryoglobulinuria: Studies of a cryo-Bence Jones protein¹

By CHESTER A. ALPER, M.D.

Over three decades ago Wintrobe and Buell (21) described the first cryoglobulin, a serum protein which precipitated in the cold. Since this observation was made, numerous examples have been reported, the majority occurring in the serum of patients with multiple myeloma or Waldenström's macroglobulinemia. In most of these cases the cryoprecipitate or cryogel has been shown to consist of a γ G or γ M protein or a complex of γ M protein with normal γ G (5). Thus cryoglobulinemia is a form of what Waldenström has termed gammopathy (19) usually of the monoclonal variety (20).

Whereas the vast majority of reported cryoglobulins have been 150,000 or more in molecular weight with sedimentation constants of 7S or greater, one cryoprotein of 3.6S recovered from the urine of a patient with multiple myeloma has been described (18). The relationship of this protein to the immunoglobulin system (6) was not de-

fined, nor did the patient's urine give a positive Bence Jones heat test.

In the present study a similar urinary cryoprotein was isolated and investigated, particularly with regard to its place among the immunoglobulins.

Materials and methods

Case report. The patient was a 59-year-old white female whose chief complaint on admission was severe back pain. On physical examination she had a blood pressure of 180/90 and muscle spasm and tenderness over the lower thoracic spine but no other abnormalities. X-ray studies revealed osteolytic lesions of the skull, ribs, and several thoracic vertebrae as well as generalized undermineralization of the bones. Abnormal laboratory findings included 11.0 gm% hemoglobin, an erythrocyte sedimentation rate (corrected) of 27 (Wintrobe method) and a blood urea nitrogen of 30 falling to 14 mg%. The white blood cell count and differential were normal as was the platelet count. Paper electrophoresis of her serum proteins revealed a total protein of 5.3 gm% with albumin 4.1, α_1 globulin 0.2, α_2 globulin 0.2, β globulin 0.5, and γ globulin 0.3 gm%. Thus, in addition to hemodilution her pattern showed possible *in vivo* hemolysis and hypogammaglobulinemia.

¹ The immunoglobulin nomenclature used here is that recommended in the WHO Bull 1964: 30, 447.

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Figure 1 Starch gel electrophoretic patterns of normal γG (the urinary cryoprotein as purified γG component) and a purified γM prepared from a patient with Waldenström's macroglobulinemia from left to right. The electrophoresis was performed at pH 8.2. The γM is characterized by its failure to enter the gel.

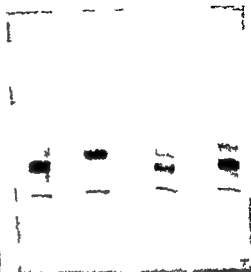


Figure 3 Polypeptide chain patterns of normal γG (the urinary cryoprotein as a γG component) and a γM macroglobulinemia protein. The pattern of the urinary cryoprotein shows material with the mobility of light polypeptide chains only. Electrophoresis was performed in starch gel with formate buffer pH 3.0 containing 8M urea and 2 mercaptoethanol.



Antibody analysis of the urinary cryoprotein. The left and center well (A) contains antiserum to kappa light polypeptide chains and the right hand center well (A) contains antiserum to lambda type chains. Peripheral wells 1 and 4 on both sides contain the antigen. The center well (A) contains a reference lambda type protein and wells 3 and 4 contain a reference kappa type protein. There is no reaction of the urinary protein with kappa antiserum but a strong precipitin band forms with the anti lambda antiserum with the spurs with the reference lambda protein.

Table I *The influence of pH and electrolyte concentration on the formation of cryogel or cryoprecipitate*¹

Phosphate Buffer Concentration	pH 5.0	pH 6.0	pH 7.0	pH 8.0	pH 9.0	pH 10.0
0.10 M		± precipitate	++ precipitate	+++ precipitate	gel	
0.10 M			semi gel	++ precipitate	semi gel	
0.05 M			gel	gel	gel	
0.01 M		gel	gel	gel	gel	semi gel
0.005 M	gel	gel	gel	gel	gel	gel

¹ All samples were at a protein concentration of 5.7 gm % and were kept at 4°C for 16 hours. The absence of an entry indicates no change in solution.

Table II *The influence of 2-mercaptoethanol (2 ME) on the formation of cryogel or cryoprecipitate*

	0.1 M 2 ME	0.05 M 2 ME	0.005 M 2 ME
0.1 M Phosphate Buffer	+ precipitate	+ precipitate	+ precipitate
0.005 M Phosphate Buffer	gel	gel	gel

All solutions at a protein concentration of 5.7 gm % and pH 8.0

electrophoresis using an anti lambda chain antiserum, each of these bands gave a precipitin arc which fused with that of its neighbor in a reaction of identity. In this technique there was no material which reacted with antisera to γG , γA , γM or kappa chains nor was there any reaction with an antiserum against whole human serum. Figure 2 depicts the reactions of the urinary protein with an antiserum specific for kappa chains (left hand portion) and another specific for lambda chains (right hand portion). For comparison reference kappa and lambda type Bence Jones proteins are included. It is clear that the urinary protein is of type lambda. The failure of this protein to react in Ouchterlony analysis or immunoelectrophoresis with antiserum specific for γG , γA or γM

suggests that it consists only of light polypeptide chains.

That this was the case was supported by the polypeptide chain pattern in urea mercaptoethanol starch gel electrophoresis as shown in Figure 3. Here it is seen that whereas normal γG and the two purified serum M components consist of both light and heavy polypeptide chains, the urinary cryoprotein is made up entirely of material with the mobility of light chains.

Ultracentrifugal analysis revealed that the urinary protein had a corrected sedimentation constant of 3.4S consistent with that of light chain dimer. Although the material gave a single peak in this analysis there was some asymmetry towards the meniscus suggesting the presence of some material of lower S value.

Summary

Studies of a cryoprotein isolated from the urine of a patient with multiple myeloma showed that it consisted only of lambda light polypeptide chains and was a typical Bence Jones protein. A solution of the protein formed a gel or precipitate or remained unchanged at 4°C depending upon protein concentration and the electrolyte molarity and pH of the solution.

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The carbohydrate content of the protein was 0.9 %

When the purified urinary protein in 0.15M saline was kept at 4°C for 16 or more hours, only those solutions at a protein concentration of 3.5 gm % or higher formed gels

The effects of pH, solute molarity and reducing agent on the formation of cryogel or cryoprecipitate are shown in Tables I and II. Thus, this protein would either gel or precipitate in the cold and which, if either occurred, was influenced by several factors. Gel formation occurred maximally at pH 9 and was favored by low ionic strength. Precipitate formation appeared to be promoted by high ionic strength and a pH of 7-8. It appeared that 2-mercaptoethanol had no effect on cryogel or precipitate formation.

Discussion

All previously studied cryoglobulins have been shown to consist of complete immunoglobulin molecules or complexes of complete molecules (5). Classical Bence Jones proteins are known to consist of only a portion of the complete molecule, the light polypeptide chains (3). The present studies indicate that it is possible for a protein consisting only of these light chains to be a cryoglobulin. They also suggest that molecular size is not an essential factor in cryoprecipitability.

In all respects the protein reported here was a typical Bence Jones protein at least in terms of the characteristics studied (1, 3, 7, 9). The absence of visible non-immunoglobulin components on starch gel and starch gel immuno-

electrophoresis strongly suggest that cryogel and cryoprecipitate formation were properties of the protein itself and not of some contaminant. This is further supported by the low carbohydrate content (2) of the purified protein.

The phenomena of cryoprecipitation and cryogel formation are not well understood. The work of Franklin and co-workers (5) suggests that weak non-covalent bonds are formed in the cold leading to aggregation or gel formation. The present studies support this concept in that the gelling of the Bence Jones protein was promoted by low molarity of electrolyte and high concentration of protein. The fact that neither maximal gelling nor precipitation occurred around the isoelectric point of the protein (perhaps pH 6-7 to judge from its mobility on paper electrophoresis at pH 8.6) suggests that the phenomena require particular amino acids in a specific steric arrangement to be ionized or not ionized and are not reflections of simple insolubility. Since the same protein behaved as either cryoprecipitate or cryogel, the close relationship of these two forms of cryoprotein is suggested.

In view of the conditions necessary for gelling or precipitation to occur, it seems highly unlikely that the patient could have been affected in any way by the curious properties of her urinary M component. However, had the urine concentration of her Bence Jones protein been higher and had the conditions for gelling or precipitation been somewhat different, the clinical consequences might have been catastrophic.

Summary

Studies of a cryoprotein isolated from the urine of a patient with multiple myeloma showed that it consisted only of lambda light polypeptide chains and was a typical Bence Jones protein. A solution of the protein formed a gel, a precipitate or remained unchanged at 4°C depending upon protein concentration and the electrolyte molarity and pH of the solution.

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Fig 1 Case 1 Flaming plasmacells



Fig 2 Case 1 Flaming plasmacell in electron microscope

with hypogammaglobulinemia with almost total absence of IgA and absence of IgM. This shows that the flaming cells plasmocytoma is not always accompanied by IgA hyperdysproteinemia. One might think that the IgA protein remains in the cytoplasm without passing into the circulation but the IEph of bone marrow homogenate treated with ultrasounds (where presumably the cells have



Fig 3 Case 1 Immunoelectrophoresis
N—normal serum
P—serum of the patient

been broken) has not exhibited any difference from the blood serum.

The cytochemistry shows that the flaming zones are not pyroninophilic and are almost completely PAS negative. No BJ exists in the urines.

From this it can be deduced that 1) the correlation between flaming plasmacells and IgA dysproteinemia is not obligatory. 2) The flaming plasmacells of this case seem to contain degenerate matter which has lost many of the characteristics of the hemoproteins.

Case 2 — Fa Lina female 36 years old kept in the University Medical Clinic of Firenze from 13 to 26-6-1963.

Clinical course

Subject obese for 2 years lumbar sacral pains. Slow development sensitive to therapy with prednisone (Lack of news of present state).

Skeleton X ray

Fractures and multiples zones of osteolysis.

Sternal biopsy

a) May Grumwald Giemsa marrow rich with total invasion of plasmacells with wide

Unusual Morphologic and Humoral Conditions in the field of Plasmacytomas and M-dysproteinemia

By RENATO DI GUCLIELMO

The increasingly extended and detailed study of plasmacytomatous diseases and of the cases of so called M-dysproteinemia continually offers the possibility of detecting unusual new conditions, which often contribute to improving our knowledge not only of these diseases but also of certain problems of general physiology and pathology.

The classical optic microscopic cytology still retains its full importance in indicating such exceptional conditions, which will later be studied more fully with the modern techniques of the electron microscopy and of immunophysics and chemistry.

From our collection of more than 200 observations we shall refer briefly to 5 cases which seem to us particularly unusual.

Case 1 — An elderly male 63 years old kept in the Hospital of Empoli from 1/7 to 18/8 1964.

Clinical course

For 3 months pains of the ribs and lower part of the spine. Slow clinical course fairly sensitive to therapy with prednisone + urethane + anabolic drugs (The patient is still living).

Skeleton X ray

Multiple zones of osteolysis

Sternal biopsy

a) May Grunwald Giemsa marrow rich in cells 90% plasmacells nearly all "flaming" (fig. 1)

b) Unna Pappenheim the flaming zones of the cytoplasm are not pyroninophilic

c) PAS lightly diffused positivity of the cytoplasm the flaming zones are less coloured or discoloured

d) Electron microscopy corresponding to the flaming zones the cytoplasm appears dilated in enormous sacs containing amorphous matter (fig. 2)

Proteins study

a) Serum total proteins g 3/100 cc. Paper electrophoresis α_1 62 α_2 71 α_3 9.3 β 12.5 γ 9.3% Immunoelectrophoresis (ILph) IgG slightly diminished IgA traces IgM absent (fig. 3) ILph of bone marrow homogenate treated with ultrasounds (with the purpose of breaking the cells) identical with that of the serum

b) Urines negative for the research of the Bence Jones proteins ILph traces of IgG

Comments

In a case of typical bone plasmacytoma with flaming plasmacells the total serum proteins were low normal there



Fig 6 Case 3

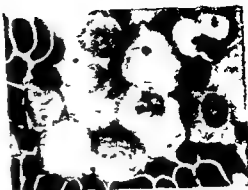


Fig 8 Case 3 Danilov's staining

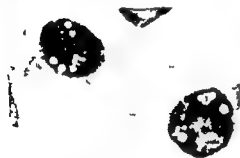


Fig 7 Case 3

Skeleton X-ray

Diffuse decalcification collapse of 2 lumbar vertebrae

Sternal biopsy

May Grunwald Giemsa marrow rich in cells with 30% plasmacells of these 61% contained characteristic round enclosures, usually multiple inside the nuclei (fig 6) surrounded by a border of dark material (fig 7) 15% showed such enclosures also in the cytoplasm 1% only in the cytoplasm 1% was without enclosures

■ PAS and Sudan Black B the enclosures did not colour

■ Danilov (for the proteic substances) the enclosures were clearly positive (fig 8)

Proteins study

a) Serum total proteins g 7.8/100 cc Paper electrophoresis A 61.4 G1 6.2 G2 11.0 B 8.5

γ 12.9% IEph quantitative diminution of the 3 Ig fractions

b) Urines albuminuria no BJ IEph slight traces of IgG notable albuminuria

Comments

In a case of bone plasmacytoma without paraproteinemia with diminution of the 3 Ig fractions and without BJ proteinuria 61% of the marrow plasmacells contained endonucleic enclosures of proteic nature. The phenomenon reached a degree and a diffusion never before noted. It is not easy to say whether the incapacity of the plasmacells to empty the proteins into the circulation can facilitate the appearance of proteic drops in the interior of the nuclei.

Case 4 — Ma Cesare male 66 years old kept in the University medical Clinic of Firenze from 7.3 to 11.4.1962

Clinical course

In 1954 fracture on the right femur consequently bone pains treated with cobalt therapy but without effect. Intense wasting tumours in the right ribs. Treated in the Clinic with large doses of prednisone (mg 150 daily X4 days 100 X3 75 X2 50 X20) urethane

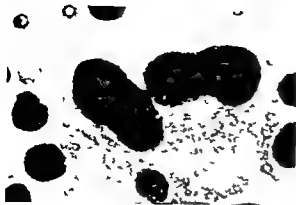


Fig 4 Case 2



Fig 5 Case 2

cytoplasms often confluent containing an enormous number of small spheres which sometimes are also on the nuclei. There are numerous giant polyploid cells with a monstrous nucleus; these also contain innumerable small spheres in the cytoplasm (fig 4 and 5).

b) Sudan black B and PAS: the spheres are negative.

c) Danielli (for the proteic substances): the spheres are strongly positive.

Proteins study

a) Serum: total proteins g 60/100 cc. Paper electrophoresis: α_1 2 α_2 5.2 α_3 1.1 β 13.9 γ 15.7%. IEph: completely normal.

b) Urines: no Bence Jones proteins.

Comments

In a case of typical bone plasmacytoma without demonstrable alterations of serum proteins and without BJ proteinuria, there are numerous monstrous polyploid cells in the cytoplasm of these as in that of the other plasmicells; there is a great number of droplets which show cytochemically, a proteic nature.

The analogy of this observation with that presented by Brucher and Meiser at the Congress of the Swiss Hematology Society in 1963 is evident.

The sole differences are represented by the proteic enclosures, present in our case but not in the Swiss one, and by the BJ proteinuria absent in our case and strongly represented in the Swiss case.

The finding of the giant plasmicells with monstrous nuclei is very rare; we consider that the cases of this type deserve to be pointed out in order to see whether there are other characteristics apart from the morphological ones which make it possible to classify them in a special variety of plasmacytomas.

Case 3 — Po Lumbio, male, 62 years old, out-patient of the Medical Clinic from November 1964 and then admitted to the Urological Clinic of Firenze in April 1965.

Clinical course

For 15 months, albuminuria without apparent causes, without hyperazotemia. For 3 months, violent lumbar pains which disappeared with anabolic drugs and an orthopedic corset. Consequently, a serious increase of the azotemia until the uremic coma; the patient died in April 1965 after 21 months of illness. A course of therapy with prednisone + urithane + anabolic drugs did not have any effect on the azotemia which steadily grew worse.

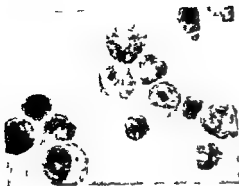


Fig 11 Case 2

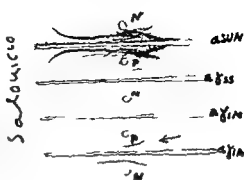


Fig 12 Case 2

y 38.2% LEph very important increase of IgA IgG and IgM normal (fig 12)

b) Urines no BJ LEph albuminuria.

Comments

In a woman 46 years old a sure hypoplastic and hypersplenomegalic leukemia was accompanied by a "major" IgA dysproteinemia. It is an absolutely exceptional combination it is not easy to understand which clone of cells produces the paraprotein unless one thinks of the same leukemic cells which have a histioid appearance which partially reminds the cells of the reticulosarcoma.

Summary

In this paper are briefly referred a observations which can be considered exceptional.

Case 1 Bone plasmocytom very rich in flaming plasmacells without IgA paraproteinemia

Case 2 Bone plasmocytom with enormous cells which have monstrous nuclei and innumerable proteic droplets in the cytoplasm with complete normality of the serum proteins and no proteinuria

Case 3 Bone plasmocytom with plasmacells extraordinarily rich in endonucleic proteic enclosures without paraproteinemia and with diminution of the 3 Ig fractions

Case 4 Bone plasmocytom IgG with the presence in the cytoplasm of the plasmacells of innumerable crystal like enclosures

Case 5 Hypersplenomegalic histioid leukemia with "major" IgA dysproteinemia

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Brucher H., Meiser J. Schweiz. med. Wochschr. 92 1426 1962



Fig 9 Case 4



Fig 10 Case 4

(g 1.5 daily) anabolic drugs. The results were extremely good on the general condition on the anemia on the proteins (see below) on the rib tumours (which completely disappeared). Further news is lacking.

Sternal biopsy

Marrow rich in cells, the normal tissue was well preserved, there were 18% of myeloma cells. Of these, a good number showed in cytoplasm very many clear rectangular bodies which looked like crystals (fig. 9) and which sometimes were found also on the nuclei (fig. 10) (There was no cytochemical examination).

A second biopsy carried out after a month in greatly improved general and protein conditions showed an almost equal number of plasmacells (15%) but the almost total disappearance of the enclosed bodies.

Proteins study

a) Serum (8.1.1962) total proteins g 9.15/100 cc. Paper electrophoresis: A 27 (2.4) α_1 5.3 (0.7) α_2 15.2 (1.1) β 11 (1.0) γ 38.2 (3.5). Biph. IgG doubled in cathodic direction. IgA and IgM normal.

(4.1.1962, after mg. 1900 of prednisone + g 23 of urethane) total proteins g 5.7/100 cc. Paper electrophoresis: A 33.2 (1.89) M 7.8 (0.1) α_2 15.2 (0.8) β 11.1 (0.6) γ 32.1 (1.8). ILph unchanged.

b) Urines: no B₂ proteins.

Comments

In a case of bone plasmacytoma with strong dysproteinemia IgG many of the marrow plasmacells showed very many crystal like enclosures. Such enclosures which, as far as I know, have never been noted almost disappeared after a period of intense therapy with prednisone and urethane, a therapy which, at the same time provoked a very important diminution of the paraprotein.

Case 5 — Greek woman 40 years old admitted to the University Medical Clinic of Thessaloniki (Greece) in 1962.

Clinical course

The patient suffered for some months from a form of histiocytoma with hyperleucocytosis and enormous splenohepatomegaly. Skeleton X-ray normal.

Sternal biopsy

Total substitution of normal marrow with histioid granulous cells (fig. 11) identical to the cells present in the peripheral blood. There it was not possible to find plasmacells.

Proteins study

a) Serum total proteins g 9.1/100 cc. Paper electrophoresis: A 30 α_1 5.1 α_2 8.3 β 13.1



Fig 11 Case a

Salomero

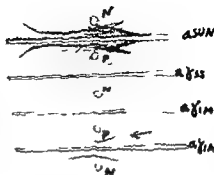


Fig 12 Case a

γ 38.2% IEph very important increase of
IgA IgG and IgM normal (fig 12)
bi Urines no BJ IEph albuminuria

Comments

In a woman 46 years old a sure hypercellular and hypersplenomegalic leukemia was accompanied by a "major" IgA dysproteinemia. It is an absolutely exceptional combination. It is not easy to understand which clone of cells produces the paraprotein unless one thinks of the same leukemic cells which have a histioid appearance which partially reminds the cells of the reticulosarcoma.

Summary

In this paper are briefly referred a observations which can be considered exceptional.

Case 1 Bone plasmacytoma very rich in fibrin, plasmacells without IgA paraproteinemia

Case 2 Bone plasmacytoma with enormous cells which have monstrous nuclei and innumerable protein droplets in the cytoplasm with complete normality of the serum proteins and no proteinuria

Case 3 Bone plasmacytoma with plasmacells extraordinarily rich in endonucleic protein enclosures without paraproteinemia and with diminution of the 3 Ig fractions

Case 4 Bone plasmacytoma IgG with the presence, in the cytoplasm of the plasmacells of innumerable crystal like enclosures

Case 5 Hypersplenomegalic histioleukemia with "major" IgA dysproteinemia

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A Case of Myelomatosis with Normal Colloid Osmotic Pressure in Spite of Extremely High Serum Protein Concentration (Hyperviscosity syndrome due to aggregation of myeloma globulin molecules?)

By MOGENS BJORNEBOE and K. BIRGER JENSEN

In many cases of myelomatosis the serum protein concentration is so high that it is hard to understand how the body is able to maintain a normal colloid osmotic pressure. The *calculated* pressure ranges far beyond normal levels.

By experimentally induced hyperimmunization in rabbits extremely high concentrations of γ -globulins can be produced (1). Examination of the plasma volume in these animals showed that the volume increases concurrently with the γ -globulin concentration and consequently it is justifiable to presume that the body strives to maintain a normal colloid osmotic pressure by means of this mechanism (2, 3). Besides, direct measurement of the pressure in samples of serum from these animals, using Tybjerg-Han-

sen's osmometer, reveals this pressure to be normal, in spite of great variations in the concentration of γ -globulins (4). By studying more comprehensive series of patients with myelomatosis in whom measurements of the plasma volume and the concentration of myeloma-protein are made it is possible to show that no correlation exists between these two values (5, 6).

Hence an increase in the plasma volume cannot be the only mechanism of control of the colloid osmotic pressure in this disease.

A study of the case reported below suggested to us that in aggregation of globulin molecules may contribute to the control of the colloid osmotic pressure in myelomatosis.

Case report

Supported by grants from the King Christian X Foundation and Vera and Carl Johan Michaelsen's Foundation.

A 51 year old man was admitted to this hospital on October 19th 1964 and died here on November 6th 1964. Apart from minor ill-

nesses he had been in good health until March 1962 when he was treated in his home with penicillin because of pneumonia. In July 1964 and shortly afterwards he had two episodes of pneumonia and was again given penicillin. A subsequent chest X-ray showed infiltration in the right lung and he was admitted to the department of chest surgery of this hospital. During his stay there he had severe epistaxis and when anaemia, proteinuria and arterial hypertension were revealed he was transferred on the 26th of October to medical department B for further study. He said that he had for some time been troubled by fatigue, dyspnoea on exertion and dizziness. Physical examination disclosed an enlarged liver extending 8 cm below the costal margin but no enlargement of the spleen and no palpable lymph nodes. Blood pressure was slightly elevated. Ophthalmoscopy on October 28th disclosed normal discs, distended veins without segmentation, numerous haemorrhages fan-shaped or in streaks and some "woollen" exudates. The ophthalmologist described the findings as typical for Waldenström's macroglobulinaemia.

On October 30th he complained of severe headache and pains in the finger joints and on November 4th of low back pain. He became increasingly sleepy, complained of impaired memory and reduced ability to concentrate. He became progressively weak and dropped things he had in his hands.

On November 5th he developed pneumonia with a slightly elevated temperature. In spite of tetracycline therapy he died on November 6th. Raynaud's phenomena or acrocyanosis were not observed during the hospital course.

Clinical and laboratory investigations

Weight 8 kg Height 1.72 cm.

Blood pressures were 160/100, 160/100 and 160/110 mm Hg.

Serum protein studies

Paper electrophoresis (on October 31st) total protein 18.6 g per 100 ml, albumin 18.2 g, 0.23 α , 0.36 β , 0.33 γ plus γ globulin 0.47 and an intermediary γ component in the γ area of 1.5-4 g per 100 ml.

Immuno electrophoresis¹ (on November 6th) revealed a γ -paraprotein with slow and intermediary mobility, reduced IgA, IgM and albumin, slightly elevated α_2 macroglobulin. A tendency to aggregation into bigger units was demonstrated. The cold agglutinin titres were normal.

By ultra centrifugation analysis (on November 26th) of serum diluted to a total protein value of 1.4 g per cent, 20.9% of the protein showed 41 Svedberg units, 78.2 per cent showed 61 and 0.6% showed 16.6 Svedberg units.²

Plasma volume determinations with ¹²⁵I labelled IgG globulin 0.020 ml, i.e. 84.4 ml per kg (normal value 40.2 ml per kg) (7).

Colloid osmotic pressure in plasma kindly determined by P. Ingerslev, M.D., the Department of Clinical Physiology (according to the method of Tybjaerg Hansen) was 47 cm of water (normal range 34-46). The calculated pressure according to (4) was 61 cm of water.

Lumbar puncture (on November 4th) pressure 240/110 mm of water. The cerebro spinal fluid contained 2 white blood cells and 17 red blood cells per c.mm, glucose 78 mg per 100 ml. Immuno electrophoresis of spinal fluid (on October 29th) was normal and revealed no signs of paraproteinuria.

ESR varied between 140 and 154 mm/hour. Haemoglobin 8.4-8.2 g/100 ml. On October 27th red cells 2.18 mil/pl, haematocrit 20 per cent, MCV 92 ml, MCHC 32 g/100 ml, platelets 24,000-10,000 per μ l. White cells 9,200 per μ l. Differential count: 42 neutrophils, 1 eosinophils, 48 lymphocytes, 1 monocyte and 1 per cent plasma cells. The red blood cells showed pronounced agglutination which made the counting difficult. This agglutination tendency disappeared by heating to 37°C.

Alkaline phosphatases 38-36 μ mol/l, strong, units per 100 ml (normal range 3-10). Basal metabolic rate (on the 3rd and 4th of November) 112 and 119 per cent. SGPT 1.4 mU per hour per litre (normal range <1.8).

¹ kindly performed by J. Clausen, M.D.

² We are grateful to B. Månson, cand. pharm., who carried out this test.

Thymol turbidity 0.71 (normal range <0.13) Serum bilirubin 0.6 mg per cent Prothrombin 62 per cent Serum calcium (on November 5th) 11.1 mg per 100 ml Serum phosphorus 4.5 mEq per 100 ml Arterial blood (on October 31st) oxygen saturation 93 per cent pH 7.44 standard bicarbonate 23.5 mEq/litre pCO_2 36 mm Hg Serum chloride 101 mEq/litre Serum sodium 130 mEq/litre Serum potassium 3.9 mEq/litre Serum haemoglobin 46 mg per 100 ml (normal range 18—196) Red blood corpuscle osmotic resistance was lowered Serum B_{12} 360 pg per ml Serum creatinine 1.8 mg per 100 ml Creatinine clearance 63 and 74 ml/min The Sia test was positive

Urine examination showed proteinuria 0.4 per cent Bence Jones protein no glycosuria Microscopic examination of urine revealed slight haematuria

Bone marrow aspirate (on October 28th) showed 34 per cent of plasma cells of abnormal structure with some bizarre types characteristic of myeloma

Blood culture (on November 6th) was negative is also culture from the spinal fluid

The electrocardiogram was normal

X-ray films of the lungs taken on July 24th September 22nd and October 21st revealed infiltration in the right upper lobe Films taken on November 5th showed numerous small infiltrations in both lungs

Intravenous pyelography was normal

Neurological examination on November 6th led to the suspicion of a paresis of throat and tongue L.L.G. was abnormal with low frequency activity especially frontally

Post mortem examination revealed confluent bronchopneumomas in both lungs Microscopic examination showed intense plasma cell infiltration in the spleen the lungs and in particular in the bone marrow

Discussion

The present case of myelomatosis is characterized by an extremely high concentration of serum protein The colloid osmotic pressure, as calculated

on the basis of the concentrations of albumin and γ globulin in serum ranges considerably beyond normal limits, whereas measurement of the actual pressure gives almost normal result (47 cm of water) This discrepancy may be explained by assuming that an aggregation of the myeloma globulins has taken place In this connection it is of interest to observe that in many respects the clinical picture in this case resembles the hyperviscosity syndrome which has recently been described by Smith et al (8), viz (1) bleeding, in particular from the mucous membranes of nose and mouth, and (2) disturbed vision, dilatation and segmentation of retinal veins, round haemorrhages papilloedema To these may be added (3) weakness, fatigability, (4) peripheral oedema, decreased pulse pressure, (5) vertigo electroencephalographic changes syncope and convulsions and (6) distension of dependent veins and capillaries, dependent plethora

Our patient exhibits several symptoms which are similar to those observed in the above patients epistaxis, haematuria typical retinopathy the finding of aggregations of paraprotein by immuno electrophoretic studies pronounced rouleaux formation in the blood smears electroencephalographic changes and peculiar neurological findings

In this connection it should be mentioned that as early as in 1944 in his first description of Waldenström's macroglobulinaemia Waldenström described these findings to hyperviscosity (9) Ott mentions in 1956 that the cal

culated colloid osmotic pressure differs markedly from the directly measured pressure in myelomatosis the same observation as we have made. However, he does not ascribe this finding to aggregation of the protein molecules (11).

True enough we did not succeed in demonstrating this aggregation by ultracentrifugation but this may be due to the fact that the serum was extremely diluted prior to examination.

Therefore we find it justifiable to assume that in our case of myeloma toxa the colloid osmotic pressure is kept down by a combination of an increase in the plasma volume and an aggregation of myeloma globulin molecules.

In addition it is only natural to explain the different mechanisms of control in the unimmunized animals and in the patient with myeloma by pointing to the fact that identical or almost identical molecules are polymerized much more easily than non identical molecules (10). Myeloma globulins are characterized by being homogenous whereas immunoglobulins produced by immunization are composed by several different types of γ globulins.

Summary

A case of myelomatosis with a concentration of γ globulin of 20 g per 100 ml is reported. The colloid osmotic pressure measured is 47 cm of water whereas the calculated value is 61. The plasma volume is 64 l ml/kg. It is postulated that the patient's colloid osmotic pressure is maintained at normal values by means of a combina-

tion of increased plasma volume and aggregation of myeloma globulin molecules. This hypothesis is substantiated by the fact that the clinical picture in this patient in certain respects resembles the "hyperviscosity syndrome" described by Smith et al.

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Fig 1 Microscopic appearance of the tumour removed from the skull Hematoxylin and eosin staining Magn $\times 100$

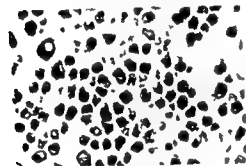


Fig 2 Plasmacell like tumour cells in the preauricular tumour Hematoxylin and eosin staining Magn $\times 1000$

those plasmacells present showed a normal appearance definite increase in eosinophils and lymphocytes Erythrocyte sedimentation rate after one hour a mm

Serum proteins total protein 7.34 gr % (albumin 60.7 % alpha 1 globulin 2.8 % alpha 2 globuline 8.0 % beta globuline 9.5 % and gammaglobulin 13.0 %) With a rabbit anti human IgM serum, no precipitation line was seen in double dimensional agar diffusion plate (Ouchterlony) with a dilution of the patient's serum 1:10

Serum value for alkaline phosphatase 182 units (King Armstrong) acid phosphatase 1.61 (King Armstrong) thymol turbidity 0.7 U sodium 140.0 milliequivalents per 1000 ml potassium 4.9 m.equ. per 1000 ml and chlo-



Fig 3 X ray of the right knee showing the mottled appearance

ride 96.6 m.equ. per 1000 ml Blood urea 40 mgr % creatin 0.58 mgr % with a clearance of 112 ml/minute Rowntree 38.5% (after two hours) Weakly positive reaction for anti nuclear factors using an immuno fluorescent technique

Reactions of Weinberg Casoni and Von Pirquet negative VDRL negative

Urinanalysis was normal

No occult blood or worm eggs in the faeces

No clinical diagnosis could be made at this time The pre auricular tumour was removed and showed the unmistakable appearance of a plasmacytoma (fig 2) Skin or bone tissue were not present in the tissue removed The tumour was divided into a few large lobules separated by broad bands of connective tissue This distribution was suggestive of lymphoglandular involvement but as there was no normal lymphoid tissue present this can not be decided for with certainty

One year later the patient was admitted to another hospital for an infiltrate in the right lung due to a Staphylococcus aureus infection He recovered after treatment with penicillin and streptomycin

About this period he started complaining about his right knee A progressive swelling developed in the right condyle of the femur

X rays of this area showed a mottled appearance (fig 3)

2 months later biopsies were taken from this lesion and from another one in the right tibia Both were compatible with a diagnosis

A Patient with Atypical Multiple Myeloma

By A. J. VAN DER GRIENT and E. J. EBELS

The history of this patient relevant to the condition to be described started five years ago. A man aged 52 years was admitted to the Department of Neurosurgery of this hospital with signs of herniation of the brain stem. Although he had noticed a slowly increasing swelling of the cranium for some years he had been well until one month before admission. At that time he started complaining of nightmares, forgetfulness and headache. He vomited once, had fever up to 39°C and was sleepy. One day before admission frequent vomiting started. The patient was found to be drowsy and dehydrated. At clinical examination the blood pressure was normal, there was no papilloedema and there were no signs of meningeal irritation. At palpation a subcutaneous mass was present in the left frontal area which was continuous with or adherent to the skull. X-ray investigation showed bone destruction suggestive of tumour infiltration. At operation (Prof C. H. Lenshoek) a golfball sized tumour was found adherent to the dura and infiltrating the overlying skull. It was considered to be a meningioma and removed as fully as possible. At histological examination a cellular tumour was seen with more or less oval cells loosely arranged in fields separated by a mostly delicate stroma. The nuclei were round, sometimes eccentrically situated and very often showed bizarre clumping of chromatin (fig. 1). The possibility of an undifferentiated plasmocytoma was considered but rejected (partly on the ground of negative pyronin staining

of the cytoplasm — it became apparent afterwards that our methyl green pyronin staining at that time was technically unreliable). The possibility of an atypical "cytoplasmic meningioma" was suggested.

The material was also seen by Prof Zülch in Cologne who suggested the possibility of a metastatic tumour, a plasmocytoma and leukaemic infiltration. We then asked Prof Dorothy Russell in London for her opinion. She thought a plasmocytoma most likely but also considered the possibility of a reticulo sarcoma.

Two years later a tumour developed in the region of the left mammary gland and it was removed surgically. Histological examination showed a non-encapsulated cellular tumour spreading within the fatty tissue of the mammary gland which itself showed the aspect of a mammary carcinoma. The cellular aspect was similar to that of the first tumour but by now some unequivocal plasmacell-like elements were present.

Shortly afterwards a chestnut sized tumour developed in the left preauricular region. At this time the patient was investigated clinically in the Department of Medicine. Physical examination was normal. X-rays of the bones did not show any abnormality. Laboratory

Blood haemoglobin 14.8 gr % leucocytes 7800 (16% eosinophils, 29% neutrophils, 31% lymphocytes, some of which had the appearance of young types), 1 monocyte. Sternal puncture: no increase of plasmacells.



Fig VI Supra inguinal tumour

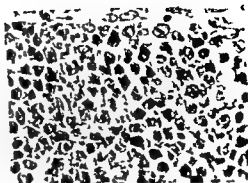


Fig VII Histological aspect of the supra inguinal tumour showing poorly differentiated cells Magn 700X

phoresis showed marked reduction of IgM and IgA globulins with a paraprotein of the IgG class. On ultracentrifugation there was a marked increase of a $\alpha_2\beta_2$ globulin presumably caused by the IgG paraprotein. An immunological search for IgG H chain paraproteinaemia was negative. After injection of 2 mgr bromsulphalein per kg body weight 20% was retained. Urinalysis was normal. No Bence Jones protein could be detected. Again no worm eggs were found in the faeces.

Bone marrow examination at this stage revealed out of 100 nucleated cells 41 (82%) lymphoid plasmacells, 3 (6%) plasmacells, 36 (72%) eosinophilic leucocytes.

The supra inguinal tumour was removed (Fig. VI). Histological examination showed a very cellular tumour with many features of those removed from the mammary and preauricular regions but with less differentiation of the cells into plasmacell like elements. It was diagnosed as a poorly differentiated plasmacytoma (Fig. VII) on the same grounds as in the biopsy from the preauricular tumour. The appearance of the tumour was suggestive of lymphoglandular involvement but here too this remained conjectural. Skin was not present in the biopsy.

In view of the multiple bone defects as seen on the X ray pictures the histological appearance of the tumours removed and the presence of an IgG paraprotein with a strong decrease of IgG and IgM globulins the diagnosis of multiple myeloma was made.

The patient was treated with melphalan which caused some subjective improvement and some regression of the swelling of the knee. The sedimentation rate of erythrocytes fell to 38 mm after one hour and 78 mm after two hours. Six months afterwards a rapidly growing tumour developed in the right testis as well as infiltrates in the right upper leg and underneath the left eye. Hemi castration was advised but was refused by the patient. X ray therapy caused some regression of the tumours. The general condition gradually deteriorated and the patient died five years after the first admission. Autopsy was refused.

Discussion

The first symptoms of the present illness of this patient were those of involvement of the central nervous system due to a large poorly differentiated plasmacytoma which originated either from the skull or from the dura mater. Later on multiple extra medullary plasmacytomas developed followed by the occurrence of multiple bone lesions. In the first stages serum protein values remained normal later on there was IgG para



Fig 11 X ray of the arms, showing lesions in the left ulna

of plasmocytoma (Prof J I Hampe University of Amsterdam)

As the patient's general condition deteriorated he was once again admitted to our department (about 4 years after the beginning of this history)

By now he was in a fairly bad condition. Examination showed multiple haemangiomas of the skin. Lymph nodes were not enlarged at palpation. In the abdominal wall above the left inguinal region a chestnut sized tumour was present, adherent to the underlying structures but not to the skin. Liver and spleen were not enlarged (as they never were throughout the illness). X ray investigation showed extensive bone lesions to be present in the left tibia, left ulna, left radius, right femur and right tibia (fig 11).

The blood pressure was 110/80 mm.

Laboratory investigations

Blood haemoglobin 11.2 gr % Packed red blood cell volume 34 ml/100 ml erythrocyte sedimentation rate after one hour 115 after two hours 132 mm (Westergren) leucocytes 7300 (2% basophils 16% eosinophils 49% neutrophils 27% lymphocytes 0% monocytes). At another occasion even 46% of the leucocytes consisted of eosinophils! Thrombocytes 20,000/mm³. Analysis of blood clotting showed no abnormalities. The tourniquet test

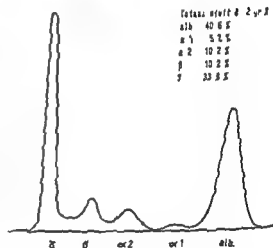


Fig 1 Electrophoretic pattern of serum proteins

was strongly positive. Alkaline phosphatase 19 IU (King Armstrong). Acid phosphatase 0.8 U (King Armstrong). Urea 35 mg % Total bilirubin less than 1 mg %. Calcium 8.4 mg % Phosphorus 2.6 mg % Serum glutamate oxalate transaminase 3 U Serum glutamate pyruvate transaminase 2 U Lactate dehydrogenase 175 U Total lipids 700 mg % Serum proteins 8.12 gr % of which albumin 40.6% alpha 1 globulin 5.2% alpha 2 globulin 10.2% beta globulin 10.2% and gamma globulin 23.8% (fig 1). Immuno electro

to the small lymphocyte as being the precursor of the plasma cell (for a survey vide Gowans and MacGregor 8). In that case we must assume a local derangement of plasmacells, or their immediate precursors as the initial event in the occurrence of plasmocytomas. As to extramedullary plasmocytomas the theoretical possibility of course remains of their being metastases of a bone lesion. We think that there is little evidence in favour of such an explanation in our case.

For the eosinophilia in this patient no ready explanation is available. Unexplained eosinophilia occurs in rare cases of multiple myeloma. Snapper et al. (16) described three patients with respectively 20%, 32% and 46% eosinophils. Mandema (13) in the bone marrow examination of 36 patients found more than 10% eosinophilic cells (as percentage of the nucleated cells of the bone marrow) in 4 cases.

As long as the cause and nature of the eosinophilic reaction in general remain enigmatic every theory to explain the combination of multiple myeloma and eosinophilia must be speculative. The more so as every explanation should at the same time account for the occasional combination and its very rarity. We may perhaps consider eosinophilia to be an expression of some sort of antigenic stimulation. In multiple myeloma either the hypothetical antigenic stimulus considered to be the (a) cause

of the tumour process itself (for the literature vide Ten Berge, 18), or the antigenic properties of the paraproteins produced by the tumour may be factors that in some cases lead to eosinophilia as the expression of sensitisation.

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1937 and the gel filtration 1959 each method has its disadvantage. Due to the high lability of many biologically important bindings against changes in pH, temperature and the ionic strength and/or the absence of possible competitive binding serum proteins in the isolated fraction all precipitation methods for investigations of bindings are to be looked at very critically.

Before we go further into the transport processes of bilirubin we will briefly characterize the transport milieu in which bilirubin itself moves. Under "transport milieu" (15, 16) is meant that mobile milieu in which substances and biological units are contained and which through transitory bindings serve the transport processes as such. The substances to be conveyed in the blood totally or partly bound to serum protein or to erythrocyte vehicles can be collected together under the heading of "transport goods". In doubtful cases it is possible to decide by the determination of the biological half life which of the complexes circulating with the blood should be regarded as the vehicles and which as the transport goods (compare the studies of Laurell (20) on iron bindings). The vehicles then are the components of the transport milieu which always have the longer biological half life.

Labile equilibrium conditions often arise between the same materials which are bound to different protein vehicles. The very size of all vehicles in the blood are completely different: the erythrocytes with diameter of 7μ

the thrombocytes with 2μ diameter then the serum proteins following (in order of size) can only be compared with one another by their molecular weights (m.w.). alpha₂ lipoproteins, with for example a m.w. of 3 400 000 then transferrin with a m.w. of 88 000, albumin m.w. 69 000 and the alpha₁ glycoproteins with a m.w. 50 000 down to 44 000.

For the transport processes the really important factor — that of surface area — amounts in the erythrocytes for an adult to 3 200 sq m, and approximately at least 200 times this for the serum proteins. The vehicle apparatus has to maintain the cells in the human body the number of which is of the order of 10—100 billion (10^{13} — 10^{14}) (24). The total length of the capillaries only in the musculature of a normal adult is according to Krogh (51) 100 000 kms. The erythrocytes must not only be considered as transporters of oxygen from the lungs to the tissue and as removers of carbon dioxide from the venous capillaries and back to the lungs.

The functional analogy between the muscular erythrocytes and the colloidal serum proteins is supported by phylogenetic observations: the earthworms and some crabs use instead of erythrocytes a chromoprotein sol with a m.w. of some millions; the function of oxygen containing vehicles is fulfilled in these animals by the colloids erythrochromes and chlorochromes —

It is known that in the human body numerous medicaments such as sulphonamides and mepacrin (98, 14, 17) but more particularly many hormones form supplementary bindings with the erythrocytes. The most carefully

The Transport of Bilirubin in the Circulating Blood and Its Pathogenetic Importance

By H BENNHOLD

Bilirubin is probably the first indigenous substrate in the body, in which it was possible to demonstrate the binding to serum proteins. This was done originally by ultrafiltration or dialysis of bilirubin containing serum (73, 74, 32). By electrodialysis and by karyophoresis Bannhold (9) discovered a special albumin bilirubin binding and based on this observation and on experiments with dyes and cholesterol (11) the conception of a general "vehiclefunction" (9, 12, 13, 14, 18). Bendich and Snapper (7) in 1933 also found bilirubin mainly bound to albumin, by using a graduated permeable ultrafilter. Pedersen and Wildenstrom (70) confirmed very reliably in 1937 this albumin binding by electrophoresis and for the first time by the ultracentrifuge.

In recent years the exclusive binding of bilirubin (C^{14}) to the albumins at least regarding transport processes was proved by free two dimensional electrophoresis (67). The older notion of plasma protein as a waste product stream (45) was not attractive to physiological research. Even Krehl

(49) in his "Pathologische Physiologie" 1930 p. 471, put forward the same supposition. It was purely from the degree of precipitation with ammonium sulphate that it was possible at that time to distinguish between albumin globulin and pseudoglobulin. Only cellular components of the circulating blood such as erythrocytes and leucocytes were credited with a transport function but not these three waste serum protein fractions, although as early as 1896 Stirling had shown the hydrophilic affinity of the serum proteins and its importance for the pathway of the water in capillaries and in the tissue.

Since then the number of separated serum proteins obtained by different methods (26, 37, 90, 93, 94) has risen to approximately 100 while the total number of substances including hormones and medicaments which are bound to serum proteins is now no longer surveyable. The most careful methods of proving bindings to serum proteins were the ultrafiltration and dialysis 1925 the electrophoresis (8, 12, 93) (1928—1932) the ultracentrifuge

1937 and the gel filtration 1953 each method has its disadvantage. Due to the high lability of many biologically important bindings against changes in pH, temperature and the ionic strength and/or the absence of possible competitive binding serum proteins in the isolated fraction all precipitation methods for investigations of bindings are to be looked at very critically.

Before we go further into the transport processes of bilirubin we will briefly characterize the transport milieu in which bilirubin itself moves. Under "transport milieu" (15, 16) is meant that mobile milieu in which substances and biological units are contained and which through transitory bindings serve the transport processes as such. The substances to be conveyed in the blood totally or partly bound to serum protein or to erythrocyte vehicles can be collected together under the heading of "transport goods". In doubtful cases it is possible to decide by the determination of the biological half life which of the complexes circulating with the blood should be regarded as the vehicles and which as the transport goods (compare the studies of Laurell (25) on iron bindings). The vehicles then are the components of the transport milieu which always have the longer biological half life.

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It is known that in the human body numerous medicaments such as sulphonamides and mepacrin (28, 14, 17) but more particularly many hormones form supplementary bindings with the erythrocytes. The most carefully

studied of such binding equilibria are the bindings of thyroid hormones to serum proteins and erythrocytes. Thyroxine thus adheres to interalbumin, to prealbumin (1) and albumin itself (35, 61), but only loosely to erythrocytes (66). Similarly triiodothyronine is bound to interalbumin and albumin, but its binding affinity to the erythrocytes is four times greater than that of thyroxine. The adrenal hormone cortisol is bound to at least three components of the transport milieu, in the first place, approx 90%, to transcortin (28, 30), an albumin glycoprotein, which occurs normally only in a concentration of 2.3—4.6 mg % (30), there is in addition, though, a considerably weaker binding to albumin, and finally an even weaker binding to the erythrocytes (63). The binding to transcortin is temperature labile, decreasing with increasing temperature (measured at 1°, 37°, and 60°), while the binding to albumin is temperature stable (85).

Quite how does bilirubin fit into this complicated transport milieu, in spite of its concentration in certain illnesses being increased to up to 50—100 times the norm?

Firstly though in order to prevent errors, two totally different transport types should be differentiated in the living organism. The first is the long-distance transport from organ to organ — 'the macro distance transport' — the motive power of which is caused directly by the pumping action of the heart and takes place in a clearly visible mobile transport milieu: the blood.

The second form of transport travels only over very short distances — "micro distance transport" — for the most part within fixed membranes, or within the cells themselves. The diffusion processes belong to this type, but even more important, the "active transports", which by the insertion of the carrier (e.g. ATP) require chemical energy. One would also include the operations of ultrafiltration and colloid osmosis in the micro distance transport phenomena, although they too are in fact partly driven by motive power from the heart.

Both types of transport processes can directly succeed one another. The change over position usually occurs at the point of contact of the circulating blood stream or the tissue liquid with the membrane of the cell or other tissue components. The transport milieu of the circulating blood guarantees therefore only the 'form of offering' of the transport goods at the surface of the fixed tissue —

Similar exchange processes between binding to albumin and binding to certain tissue elements (amyloid) can occur rapidly even when no enzymatical influence can be supported.

1) The relationship between the disappearance rate of the Congo red — normally albumin bound — and the albumin level in the blood was especially evident in the two anaalbuminaemia patients it amounted to 7% and 92% respectively in one hour instead of the normal 13—29% — With nothing but 202 g albumin given intravenously the disappearance rate was normalized (20, 69).

2) The high affinity of Congo red to the amyloid substance *in vitro* is known since 1922 (8a, 9a). It therefore is probable that in patients with amyloidosis the speed of disappearance of the dye is mainly caused by

the higher binding affinity of Congo red to the amyloid substance than to the albumins. By comparing simultaneously the Congo red concentration in the vena cubitalis and in the vena hepatica Lanai (72) showed in 9 patients with extensive amyloidosis of the liver a difference of 12.2 to 47.9 % within one passage through the liver.

The literature concerned with these processes has grown enormously from 1938 to the present day. Lit Bennhold and Ott (20) Hitzig (46) de Traverse (90) Schwick (84).

Before we continue and consider the particular characteristics of bilirubin transport we must first explain the meanings of "*Insufficiency of Transport Milieu*" and "*Transport Illnesses*" with selected examples. An "insufficiency of transport milieu" manifests itself clinically in that in fixed tissue adhesion points of second rate affinities will be occupied or additionally occupied by the transport goods in question normally fixed to the serum proteins. "Transport illnesses" are illnesses where a detectable insufficiency of one or more components of the transport milieu plays a deciding pathogenic role. An insufficiency of the transport milieu can be caused by the following conditions:

1) A congenital deficiency or even a fault in the development of one of the components of the "vehicle apparatus" (for example in the field of the erythrocytes sickle cell anaemia thalassaemia and the atypical hereditary haemolytic anaemias besides the genetic methaemoglobinemia in the field of serum proteins analbuminaemia (6 24 36 88) atransferriemia (44) and deficiency of al-

pha₁ antitrypsin (26) and other defectopathoproteinaemias

2) Acquired toxic, or other similar quantitative or qualitative damages to the vehicles. CO poisoning toxic methaemoglobinemia (as for example in nitrous poisoning), toxic anaemia iron deficiency anaemia, (damaged oxygen transport), dysproteinaemia and hypoproteinaemia e.g. protein losing gastroenteropathia, kwashiorkor (72) and nephrosis (28).

3) A pathological increased influx of certain transport goods into the blood without a corresponding possibility of adjustment of the vehicle in question. This is the case in primary haemochromatosis (15 43 99) where an uncontrolled resorption of iron in the intestine results in a permanently increased iron influx into the blood, without a corresponding increase of the transferrin level.

4) A pathological blockage in the utilization of the transport goods so that a reduction in the physiological removal and thereby a pathological accumulation of transport goods in the blood stream occurs without sufficient compensation possibilities being activated. An example of this is Wilson's disease (13) which through the blocking of the ceruloplasmin synthesis and by an excessive absorption of copper in the intestine leads to pathological accumulation in the blood of the only loosely albumin bound unutilized copper (76 77 92), this results in an occupation of the secondary binding sites in the tissue whereby the symptomatology of the illness is characterized (10 16).

Analogous to this is the *sideroachrestic anaemia* (42), which often results in haemochromatosis, because the blockage of the haem synthesis causes an accumulation of iron in the blood, and an insufficiency of the available transferrin.

5) A decompensated transport milieu, through an influx of foreign substances, which by competition displace bound substances from sites on the vehicles. This is of considerable importance, for only the components of medicaments (23) and hormones (2) not bound to the serum proteins, are pharmacologically and endocrinologically active. The protein bound components are thus an inactive reserve. A diabetic with well adjusted tolbutamide medication can suddenly develop severe hypoglycaemia, when given sulphamide simultaneously. Both medicaments occupy the same binding sites on albumin (17, 14, 29, 83, 98), which results in competitive displacement reactions and in increasing the concentration of free active tolbutamide in the blood (23). Conversely in the test of albumin bound bromsulphalein (BSP) tolbutamide accelerates the disappearance of the dye from the blood in the liver and can therefore in liver tests lead to incorrect diagnosis (38, 39). In addition to this though tolbutamide can, through a similar substitution, sink a pathologically increased bilirubin level through displacement of bilirubin into the tissue (64). Brodie (23) has explained the antirheumatic effect of the salicylates of phenylbutazone and of indomethacin on the basis of the displacement of

the endogenous cortisole from its binding to the albumins.

The situation of the bilirubin is exceptionally complicated regarding macro- and micro distance transports.

Bilirubin results from the autonomous haemoglobin metabolism as an intermediate product. Apart from this, as soon as it penetrates into cells other than liver cells, it produces most serious injuries to the cell respiration by uncoupling the enzymatical oxidative phosphorylation (100). The transport of this highly toxic material from organ to organ must be particularly well regulated.

1 mol of albumin binds with 4 mol of non conjugated bilirubin (67). With bilirubin C^{14} it was proved in continuous flow electrophoresis that without doubt only albumin is concerned as a binder in the macro distance transports of bilirubin. Even in a case of a child with congenital non haemolytic jaundice lacking glucuronyl transferase in the microsomes of the liver cells (Crigler Najjar disease), it was found that in electrophoresis of the serum, despite of a bilirubin level of 22 mg % the curves of the albumins and the bilirubin C^{14} coincided very closely (67).

Special transport problems arise in the foetal circulation.

Schenker, Dowher and R. Schmid (78) made the following observations in guinea pigs 2 hours after the injection of unconjugated bilirubin C^{14} into the umbilical, 52—66 % of the radio activity was found in the bile of the mother, after the injection of conjugated bilirubin though only

4% The same results were found in apes (27) — If the foetus of the guinea pigs were injected with unconjugated bilirubin and at the same time additionally with albumin (21) only the bilirubin passed the placenta but not the previously binding albumin (21) The foetal bilirubin must therefore come into contact with particularly strong binding elements of the placenta which loosen the in vitro stable bilirubin albumin binding the dissociated bilirubin can then pass over into the circulation of the mother

The erythrocytes are also able to bind small quantities of unconjugated bilirubin to their lipid membranes In vitro 100 ml of washed and packed erythrocytes can reduce by absorption the bilirubin level of a hyperbilirubinaemia serum by 3 mg% Added albumin brings about a equilibrium between bilirubin bound to erythrocytes and to albumin (26) With Coombs positive erythrocytes the binding affinity of bilirubin as compared with Coombs negative erythrocytes is reduced by a half This can have some significance in the interchange transfusion with erythroblastose children the exchange of the Coombs positive erythrocytes of the baby for the Coombs negative erythrocytes of the donor can almost double the rate of elimination of the bilirubin by the erythrocytes —

Because not only the indirectly reacting but also the directly reacting glucuronized bilirubin is normally bound quantitatively to serum protein every jaundice must be a clinical symptom of an insufficiency of trans-

port milieu for bilirubin The localization of bilirubin in the tissue results from the secondary or tertiary affinities of elastica in the sclera and in the skin of the cartilage and osteoid tissue

Which of the five previously outlined conditions for an insufficiency of the transport milieu concerning bilirubin are now realized by the different forms of jaundice?

I *Deficiency of certain vehicles* In the case of missing or almost completely missing vehicles for bilirubin e.g. in intralbuminaemia (19 21 36 59 60) we find surprisingly enough no truly clinical symptoms of an actual insufficiency of the transport milieu for this highly poisonous bilirubin no jaundice nor kernicterus! — Perhaps the overloaded scarce albumin vehicles bind the bilirubin more loosely which leads to an accelerated delivery onto the liver cells like the displacing effect of tolbutamid for bilirubin bound by albumin mentioned above (p 168) It is altogether surprising that in intralbuminaemia in spite of the very multiple although of course labile binding capacity of the albumins in healthy people usually very few clinical symptoms appear Even edema is not obligatory Thus it is obvious that in this recessive hereditary anomaly various compensatory mechanisms intervene in the various organs (19 59 60 69 73) In the other cases of congenital or acquired deficiency (e.g. nephrosis (28)) no jaundice is observed It is remarkable that the loss of 90% of circulating albumins with such a multiplicity of

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defect in Gunn rats. The under developed glucuronization apparatus in premature babies is an essential factor in the outbreak of a serious illness the kernicterus. In some pregnancies hormones are developed which in the foetus and also in the infant during breast feeding impedes the glucuronyl transferase (33-54) in this way serious jaundice and eventually kernicterus can occur (3-10). The antibiotic novobiorin similarly impedes the glucuronization and so does chloramphenicol (20). The excretion hindrance of the glucuronized bilirubin is interpreted as the cause of the congenital Dubin Johnson syndrome (31) and also of the Rotor syndrome (75). Also jaundice after chlorpromazine is supposed to result from an excretion hindrance in the cell membrane. Billing (22) injected bilirubin intravenously (2 mg/kg body weight) to 7 normal subjects and 22 patients with conjugated and unconjugated hyperbilirubinaemia. The curves were fitted by 2 exponential terms. In numerous cases she could localize the damage either on the membranes where bilirubin enters or where it leaves the cells or in the microsomes. The further transformation and the pathway of bilirubin in the bile ducts in the intestine and in the enterohepatic circle are not within the scope of this paper. In the lamb which has a relieving role in bile obstruction bilirubin is bound to the albumins too (97).

1. *Decompensated transport milieu by influx of competing substances.* In transports with temporary binding to proteins obviously displacement pro-

cesses frequently play an important role. These can be localized either in the circulating plasma proteins or intracellularly in the organs which carry out the further treatment or elimination. It is obvious that combinations of both types are possible. The localization of these displacement processes can be ascertained through the determination of the disappearance rates first by intravenous application of each single competing substance and then by simultaneous application. If the competition has its seat solely in the plasma that is to say within the sphere of the macro distance transports the displaced material produces by occupation of the binding sites on the serum proteins a sudden decrease in the concentration of the removed material. Examples are the accelerated disappearance of intravenously injected bilirubin C^{14} by additional injection of salicylate (82) acceleration of the disappearance curve of bromsulphalein through addition of tolbutamide, carbutamide or sulphonamides (44). — If however the place of competition lies within the domain of the admitting liver cells or the elimination organs thus within the sphere of the micro distance transport then the displaced material produces an elimination hindrance from the blood stream this leads to a reduction in the concentration decrease or even a slow back into the vessels. Examples of this are a check in the BSP disappearance curve through the simultaneous application of rose bengal (25, 62) of bilirubin (64) or of sodium dehydrocholate (23). It is only possible

bindings to the transport goods, is so well compensated by the organism. Only if certain exceptional transport loading occurs, e.g. through intravenous injection of medicaments usually bound to albumins, serious complications can arise (17). — In contrast to this though in *atransferrinaemia*, the almost complete deficiency of the — normally 6.6 g — circulating transferrin, has serious clinical consequences (44). There appear everywhere in the different organs serious damages through pathological iron deposits, because of the failure of the specific transport milieu without compensating mechanisms. In the *analbuminaemia* cases we found a low bilirubin level (only traces). Will this reduced quantity of bilirubin be still adequately bound to the circulating albumin?

II *Qualitatively damaged vehicles*
We do not know of any toxic influence which might modify the albumin qualitatively, so that its binding capacity pro g albumin would be decreased (analogous to carboxy haemoglobin in the erythrocytes).

III *A pathologically increased influx of bilirubin* appears in all illnesses with a clearly increased decomposition of the erythrocytes. It can be genetically conditioned, as for example in the constitutional haemolytic jaundice, in the hereditary non spherocytic anaemia, in thalassaemia, in sickle cell anaemia, and finally to a certain degree, in pernicious anaemia.

The macro distance bilirubin transport by the albumins — in spite of the extensive bilirubin production — in most cases capable to avoid bilirubin

intoxications. In illnesses mentioned above the macro distance transports taking place in the vascular system, are relieved through the joining of the intrahepatic, micro distance transports and through the large reserve of the intracellular treatment apparatus according to Sherlock (86, 87), the bilirubin content of the blood increases for example by only 2–3 mg %, while through haemolytically active poison, the influx of bilirubin rises to 1500 mgm per day, that is to six times of the normal.

IV *A pathological blockage to the further treatment of the transport goods* bilirubin is found under following circumstances: on the way into and through the liver cells, bilirubin has three important stations to pass: 1) the membrane, 2) the microsome with the enzymes for the glucuronization, 3) the exit point of the glucuronized bilirubin through the cell membrane into the bile ducts. Sherlock and Billing (87, 22) have collected descriptions of certain illnesses with damages of the cell membrane, as well as of differentiable organelles inside the liver cells. In the congenital Gilbert's illness (34) the entry of the bilirubin into the liver cells through the cell membrane is impeded. It also appears that mule fern extracts are able to hamper the admission of bilirubin into the liver cells.

In the second stage the microsomes the glucuronization of the bilirubin takes place. Congenital enzyme defects e.g. Crigler-Najjar syndrome (27) occurs in the microsomes. This corresponds exactly with the enzyme

treatment with saheylate or sulphonamides (81)

Odell was the first to draw therapeutic conclusions from these clinical observations and experiments by albumin infusions. He succeeded in displacing bilirubin out of the tissue. These albumin infusions have particular value when they are connected 30 minutes later with an exchange transfusion so that part of the bilirubin drawn out of the tissue into the blood stream can be removed. Koch and Rind (48) have injected intravenously selected cases of premature babies with serious hyperbilirubinaemia with 1 g of albumin per kg body weight after a "reflux" time of 20 minutes they attached an exchange transfusion. The largest quantity of eliminated bilirubin could in one exchange amount to 168 mg. The number of cases where second and third exchange transfusions were necessary could be decreased by this treatment from 27% to 7%.

From these examples of kernicterus we can see the damage to the total organism which can arise when the transport milieu becomes insufficient for such toxic transport goods as bilirubin. We recognize further what great significance the displacement processes can have for the micro distance transports — Also in vitro presence of bilirubin considerably limits the binding capacity of albumin for sulphonamides (83) — A hyperbilirubinaemia just as severe hypalbuminaemia can under certain circumstances disturb the optimal peripheral distribution of medica-

ments and the delivery to the tissue and the organs. Thus refers particularly to intravenously applied medicines in which the speed of injection plays an important role in the initial delivery of transport goods to the tissue.

If unexpected side effects of medicines occur the clinician should consider the possibility of a transport insufficiency. To exclude such factors however not only the absolute albumin contents of the blood should be determined but also competition processes by other albumin bound material must be taken into account.

With the complexity of the transport milieu in the blood it should be considered whether the illnesses which are decisively conditioned by insufficiencies of the vehicle functions should not be withdrawn from the mass of metabolic illnesses under the heading of "Transport illnesses", this might be also be of value for the therapeutic perspective.

Summary

1 A brief survey has been given about the development of the opinions of the bilirubin bindings to the serum proteins. The albumin bindings for this highly toxic substance are of the greatest importance for bilirubin transport.

2 The idea of "transport milieu" and its insufficiency symptoms have been interpreted from some illnesses.

3 A difference has been made between the macro distance transports in the blood stream and the micro di-

to surmise the localization of these competition processes, particularly within the liver cells. These considerations will concern the transports of bilirubin through the cell membrane to the microsomes and through the excreting membranes to the bile ducts. It is possible that sodium desoxycholate impedes not only the elimination of the BSP from the blood, but, additionally, after the fading away of the desoxycholate, the dye flows back into the bloodstream. This suggests that the hindrance to further treatment of the bilirubin and also of BSP in this case, is localized near the entrance to the cell membrane. In Dubin-Johnson disease this reflux of the dye into the bloodstream was exceptionally high.

The processes on the cell membrane, and in the intracellular transports as far as to the microsomes can however after the previously described experiments, be explained with a considerable degree of probability as a consequence of a physico-chemical binding gradient [adsorption chain according to Kallée (47)] in a similar way Piller (71) has advanced suggestions about binding colloids on both sides of the cell membrane.

In the dramatic illness of Kernicterus, based on erythroblastosis in premature babies we find all the five previously mentioned causes of serious insufficiency of transport mechanism combined in the same illness.

I) The albumins as the vehicles for bilirubin are reduced compared with the full term babies.

II) The Coombs positive erythrocytes of these patients bind only half

as much bilirubin as the Coombs negative would normally do.

III) The large amount of bilirubin caused by the extensive haemolytic processes results in a very high bilirubin level in the blood.

IV) The microsomes in the liver cells and their glucuronyl transferase production are still not completely developed, so that the normal way of elimination for the glucuronized, now water soluble, bilirubin through the kidneys cannot be used.

V) The children are also endangered by the series of medicaments which should be used because of their general under development and diminished resistance. These drugs often occupy the same binding sites as bilirubin (82-83). In this way actual competition processes arise: the lipid soluble bilirubin bound to the albumin is displaced from these binding sites and finds new binding sites on the elastica of the tissue. With newborn children, however, this can take place also on the lipids of the central nervous system (65).

These medicaments are normally harmless remedies such as salicylate, sulphonamide, caffeine, benzoin and antibiotics etc. It was Silverman (89) who first established that after premature icteric children had been treated with such medicaments the percentage of those with Kernicterus rose sixfold.

In young Gunn rats with their genetically conditioned deficiency of the glucuronyl transferase and their hyperbilirubinemia, similar cerebral damage can be produced through

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2 The idea of "transport milieu" and its insufficiency symptoms have been interpreted from some illnesses.

3 A difference has been made between the macro distance transports in the blood stream and the micro di-

stance transports, which to a large extent take place intercellularly. The two types of transport often succeed each other. The albumins with their variety of binding abilities, and thereby the frequent opportunities for displacement processes have been discussed.

5 The five most frequent causes of an insufficiency of a transport milieu have been described.

6 These have been collectively demonstrated using the example of Kerner's

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III

BLOOD DISEASES

Siderocytes, Sideroblasts and Sideroblastic Anaemia

By J V DACIE and D L MOLLIN

Siderotic granules in erythrocytes demonstrable by Perl's Prussian blue reaction were first described by Grunberg (17-18) in the erythrocytes of mouse rat and human embryos and in the erythrocytes and erythroblasts of mice with a congenital anaemia (19). They were soon found however to be present sometimes in the erythrocytes of human patients who had undergone splenectomy (13). Grünberg called these cells siderocytes.

Dacie and Doniach (9) in a study of several patients with severe blood diseases i.e. congenital and acquired haemolytic anaemias after splenectomy and myelocystosclerosis showed that similar iron containing granules were present in the haemoglobinated erythroblasts of patients whose peripheral blood contained many siderocytes. They confirmed too that many of the discrete basophilic granules demonstrable by Romanowsky dyes which might be seen in the erythrocytes of patients after (for instance) splenectomy — the so called Pappenheimer bodies (10) — stained positively for iron.

Subsequently, Douglas and Dacie (14) and Kaplan Zuelzer and Mouriquand (25) studied in detail the occurrence of siderotic granules in the erythrocytes and their nucleated precursors in normal subjects and in a wide range of blood disorders. Siderocytes were not found in the peripheral blood in health although they were demonstrated in marrow reticulocytes; they were found sometimes in large numbers after splenectomy. Siderotic granules were however demonstrable in from 20—90 % of the erythroblasts in normal subjects; the granules were always small and sometimes difficult to see. They were also found in erythroblasts in a wide range of blood diseases; they were always absent however where there was iron deficiency.

Kaplan Zuelzer and Mouriquand (25) introduced the term "sideroblast" to describe an erythroblast in the cytoplasm of which siderotic granules could be detected (by ordinary light microscopy). Both of the groups of workers cited above demonstrated therefore that the presence of sidero-

tic granules in erythroblasts was a normal phenomenon, they pointed out, too, that the proportion of erythroblasts containing the granules and their number and size were increased in conditions where there was evidence of defective erythropoiesis or haemoglobin synthesis. Douglas and Dicu (14), moreover, illustrated the occurrence of large siderotic granules surrounding the nucleus in the form of a collar in a patient considered (at the time of writing) to have an "acquired defect of haemoglobin synthesis". Details of this patient (who undoubtedly had sideroblastic anaemia) were published 6 years later (10) (Case 1).

The first definitive description of a chronic refractory anaemia in which sideroblasts in the marrow were the outstanding feature was provided by Bjorkman (4). The illness in three patients in this series was benign and long continued but the fourth patient died of myeloblastic leukaemia. Since this time many more cases of "sideroblastic" anaemia have been described and it is now realized that these anaemias occur far more frequently than was at first thought likely. The pathogenesis of the sideroblastic anaemias — they comprise a group — is still poorly understood and it is the purpose of the present paper to review briefly present knowledge in this field, with particular reference to the role of the spleen in controlling the number of siderocytes in the peripheral blood, types of sideroblasts, the classification of the sideroblastic anaemias, the role of deficiencies of

pyridoxine and other vitamins in the production of sideroblasts and the relationship between sideroblastic anaemias and leukaemia.

Siderocytosis and Splenectomy

As already pointed out, siderocytes are not found in appreciable numbers in the peripheral blood in health although a small percentage (0 to 3% of marrow erythrocytes) can be demonstrated amongst marrow reticulocytes (14). On the other hand, some siderocytes seem always to be present in the peripheral blood after splenectomy, unless there is iron deficiency. The iron demonstrable in normal normoblasts and reticulocytes is iron taken into developing cells in excess of that which is immediately required for haemoglobin synthesis. This excess iron is in all probability utilized during the completion of haemoglobin synthesis which occurs during the final ripening of the reticulocyte. There is evidence to suggest that reticulocytes soon after leaving the marrow become sequestered in the spleen (in health as well as in disease) for a matter of hours or even a day or so (1) (24) and it seems reasonable to suppose that the iron demonstrable in these reticulocytes is utilized at this time for the continuing synthesis of haem. In consequence when the reticulocyte finally escapes from the spleen into the peripheral circulation too little iron is present for it to be demonstrable as granules visible by light microscopy. After splenectomy, it seems likely that the maturation of

Table 1 Types of Sideroblast

1 Normal normoblasts	small granules difficult to see
2 Normoblasts (or megaloblasts)	with more and larger granules easily visible as in haemolytic anaemia megaloblastic anaemia thalassaemia haemochromatosis transfusional siderosis
3 Pathological sideroblasts	many granules often very large rim ^o sideroblasts characteristic. as in 177 and 277 sideroblastic ^o anaemias

reticulocytes takes place in the circulating blood to a greater extent. This means that some peripheral blood reticulocytes will be siderocytes which in fact is what is found.

In certain pathological states (see below) where more iron accumulates in the developing cells than can be utilized for haemoglobin synthesis the erythroblasts and reticulocytes may contain far more and larger siderotic granules than normal. With the spleen *in situ* it seems that this excess of iron can be removed probably usually at the reticulocyte stage by a mechanism referred to by Crosby as "culling" (Crosby (8)). In Crosby transfusion experiments showed too that the spleen was capable of removing large siderotic granules from adult erythrocytes. Furthermore, histological sections of the spleen from patients with sideroblastic anaemia invariably show an excess accumulation of fine iron granules in littoral cells lining the sinusoids. On the other hand if patients whose erythroblasts contain more iron than can be utilized for haemoglobin synthesis undergo splenectomy this iron remains in reticulocytes and later in

adult erythrocytes until the end of the cell's life span because there seems to be no mechanism for its removal except the spleen. Hence the very high siderocyte counts which may be found after splenectomy in patients suffering from disorders of haem synthesis.

Three Types of Sideroblast

The term sideroblast was first introduced by Kaplan, Zuelzer and Mouriquand (21) to describe an erythroblast with iron containing granules visible in its cytoplasm. Subsequently it has been used to describe the type of sideroblast seen in sideroblastic anaemia. This is confusing but unfortunately there is no easy solution to this problem of nomenclature. The present authors recognize three types of sideroblast (Table 1). The first is the sideroblast seen in normal marrow; the iron containing granules are few in number and difficult to see; they are very difficult to photograph and are in any case only visible in a proportion of erythroblasts.

The second type of sideroblast is found in conditions in which the per-

centage saturation of transferrin is increased. They are therefore found in conditions in which the body stores of iron are increased (haemochromatosis and transfusional siderosis) or in which there is excessive hemolysis or in which there is dyshaemopoiesis (as in megaloblastic anemia or thalassemia). The granules in such cases are larger than normal and may be more numerous, they are diffusely scattered in the cells' cytoplasm and seldom if ever form a conspicuous ring or collar around the nucleus. Almost all cells are affected.

The third type of sideroblast is found in the sideroblastic anaemias. The siderotic granules are larger than normal and many are present, they may be diffusely scattered throughout the cytoplasm but in some cells at least they may form a conspicuous ring or collar around the nucleus. These are "ring" or "ring type" sideroblasts. The cause of the ring arrangement is not clear. In some cases at least the iron in this position is located in perinuclear mitochondria (2). To some extent, too, the granules may be crowded around the nucleus because of poor development of the cells' cytoplasm. Excess iron stores and a high serum iron concentration are frequent findings in these cases, too, and the effect of this is exacerbated by impaired haem synthesis leading to defective iron utilization. In some patients, however, ring-sideroblasts may be seen when the percentage saturation of transferrin is normal or only slightly raised.

The Sideroblastic Anaemias

We should like to define a sideroblastic anaemia as an anaemia in which there is clear evidence for abnormal sideroblastic development and in which some at least of the sideroblasts are of the ring type. The presence of the abnormal sideroblasts is associated with hypochromia of the peripheral blood erythrocytes but, rather characteristically, normochromic cells are present also, in varying proportion, the result is a well marked dimorphic picture. Occasionally the hypochromic cells are few and far between.

Table II Types of Sideroblastic Anaemia

Hereditary	Sex linked hypochromic anaemia
Acquired	
Primary	Refractory sideroblastic anaemia
Secondary	Myeloproliferative diseases Malabsorption syndrome rheumatoid arthritis carcinoma etc. Drugs:INH cycloserine lead, alcohol

Sideroblastic anaemia is *not* a single disorder, there are in fact both inherited and apparently acquired types. The acquired types can be separated into two groups, namely, primary (or idiopathic) cases and cases secondary to or associated with a variety of underlying diseases (Table II). Sideroblastic anaemia too, may result from the use of certain drugs, for example, patients on anti tuberculosis drugs or with chronic alcoholism may show this picture and we have recently found sideroblastic change associated with haemolytic anaemia in five patients with partial gastrectomy who

Table III Hereditary Sideroblastic Anaemia

<i>Age of onset</i> 6—30 years	<i>Sex</i> usually male
<i>Anaemia</i> characteristically positive	
<i>Anaemia</i> hypochromic often dimorphic	
<i>MCHC</i> 22—28 %	<i>MCH</i> 74—84 μ
<i>Reticulocytes</i> 1—4 %	<i>Siderocytes</i> many after splenectomy
<i>Hb F</i> absent or trace	<i>Hb A₂</i> reduced
<i>Cr T₁₂</i> slightly decreased	<i>Serum Fe</i> increased
	<i>TIBC saturation</i> raised

Partial response to pyridoxine and/or folic acid not infrequent

were taking large doses of phenacetin or Panadol (p acetamidophenol) In respect of the multiplicity of causes the situation may be looked upon as analogous to the megaloblastic anaemias where interference with vitamin B₁₂ or folic acid metabolism can be brought about in a number of ways The final result — megaloblastic change in the marrow — is however identical whatever the cause and the same seems to be generally true of the morphological changes of sideroblastic anaemias

Hereditary Sideroblastic Anaemia

This is a rare disorder but as its existence becomes better known no doubt more cases will be recognised It seems first to have been described although not at the time recognised as a sideroblastic anaemia by Cooley (6) and Rundles and Mills (34) The disorder presents as a chronic hypochromic anaemia affecting males particularly The anaemia may be of severe grade and necessitate blood transfusion

The diagnosis has generally been made in adolescence females are less severely affected and here the diag-

nosis is usually made in the course of family studies Inheritance typically follows a sex linked apparently partially recessive pattern (34) (27) The prognosis (for males) is not good and deaths have particularly resulted from thromboembolism consequent on splenectomy

The patient's illness is often misdiagnosed as an iron deficiency anaemia In reality although the blood picture in respect of its hypochromia may superficially point to this diagnosis there is no deficiency of iron On the contrary more often than not the marrow and liver and other organs usually contain a great excess as the result of previous futile iron salt therapy The main haematological findings are summarized in Table III

Hereditary sideroblastic anaemia superficially resembles thalassaemia but it can be distinguished from thalassaemia by its different mode of inheritance haematologically although the blood pictures have many points of similarity sideroblastic anaemia is characterized by subnormal amounts of Hb A (32) and by relatively low levels of Hb F There is also the important difference that in thalassaemia

ring sideroblasts are absent or present in only very small numbers. From the therapeutic point of view there is a chance of a response to pyridoxine therapy (see later), which is not the case in thalassaemia.

Acquired Sideroblastic Anaemias

Our personal experience as to the relative incidence of primary (idiopathic) and secondary acquired cases is summarized in Table IV. One third or more of the patients had the primary disease. This relatively large group of patients represents all the patients diagnosed as having sideroblastic anaemia who have been studied at Hammersmith Hospital over the last 16 years. Clinical and haematological details of the first seven primary cases have already been published (10).

Although it is true that many of our patients were referred to us because of our known interest in the disorder, it seems clear that primary acquired sideroblastic anaemia is not a rare disease, in fact it seems to be the commonest cause of refractory dyshaemopoietic anaemia of obscure origin occurring in adults, if leukaemia and aplastic anaemia are excluded. But cases will not be recognized at all frequently unless bone marrow films are stained for iron as a routine.

Primary ("Idiopathic") Acquired Sideroblastic Anaemia

The disorder most commonly affects middle aged or elderly subjects. The age range in our series is 37—86 years, the mean age on diagnosis being 70

Table IV Primary and Secondary Acquired Sideroblastic Anaemia

	No. of cases
1 Primary acquired sideroblastic anaemia	
¹ Refractory anaemia in older patients of either sex	23
Presentation other than anaemia (eg fracture pruritus pyrexia haematuria)	6
Total	29
2 Secondary acquired sideroblastic anaemia	
Rheumatoid arthritis	6
Polyarteritis nodosa	1
Leukaemia	5
Polycythaemia vera thrombocythaemia or myelofibrosis	6
Myeloma	1
Carcinoma	4
Haemolytic anaemia (hereditary spherocytosis elliptocytosis)	3
² Malabsorption syndrome and partial gastrectomy	1
Myxoedema	1
Drugs	
Isoniazid (INH) therapy	3
Alcoholism — dietary deficiency	2
³ Phenacetin Panadol (p acetamidophenol)	5
⁴ Lead	1
Imuran (azathioprine)	1
Total	29

¹ One patient developed sideroblastic anaemia eight years after being treated for six months with Neomerzole for thyrotoxicosis.

² These patients had partial gastrectomy or blind loop syndrome. There was also an associated mild haemolytic anaemia.

³ One patient had severe acute lead poisoning, the other was exposed to lead for sixteen years and developed overt sideroblastic anaemia twenty years after exposure.

years. Both sexes have been affected, 18 females to 11 males.

The patients usually have presented with symptoms attributable to anaemia extending over a period of months or even years. Often they have received previous treatment with vitamin B₁₂ or iron without effect. Physi-

cal examination is usually unrewarding. However, the spleen may be just palpable.

The peripheral blood picture is characteristic and may permit a tentative diagnosis to be made. The abnormality which if appreciated suggests sideroblastic anaemia is the occurrence of a minority of hypochromic erythrocytes amid many more or less normal looking cells. The proportion of hypochromic cells varies from case to case: they are never absent but in our experience they do not dominate the picture as they do in hereditary cases. Anisocytosis, poikilocytosis and macrocytosis are present to a variable degree. Erythroblasts are seldom seen and the leucocyte and platelet counts are usually normal. Siderocytes are typically absent or present in only very small numbers unless the spleen has been removed; then many extremely hypochromic cells containing large siderotic granules will be present.

The bone marrow is characteristically hypercellular with erythropoiesis dominant. Nearly every erythroblast contains in excess of siderotic granules and many of them will be ring forms. The marrow particles contain as a rule a great excess of iron. Typically this is mainly in the form of many small fine granules of approximately the size of the siderotic granules seen in developing erythroblasts. It appears probable in fact that the extra-erythroblast marrow iron is in large part derived from siderotic granules originating in erythroblasts or reticulocytes which have

failed to mature or from similar granules extruded from developing cells in the course of successful maturation.

Megaloblastic change (overlay) is not uncommon in some cases; this may be marked and easy to diagnose in others the changes are minimal. This is due to deficiency of folic acid not vitamin B₁₂ (see below).

Deficiency of Folic Acid and Pyridoxine

Deficiency of folic acid and pyridoxine is relatively frequent in sideroblastic anaemia; this applies probably to both the hereditary and acquired types. Amongst our series of primary cases there were 80% in which the serum folate concentration was subnormal and 75% of those who were tested gave positive FIGLU tests. Positive FIGLU tests and excessive excretion of urocanic acid were however noted in some patients with no biochemical or haematological evidence of folate deficiency. Approximately 50% excreted xanthurenic acid after a tryptophane load indicating deficiency of pyridoxine or derangement in its metabolism. The cause of the folate (folic acid) deficiency seems likely to be an increased requirement due to greatly increased although largely ineffective erythropoiesis in addition a defect in pyridoxine metabolism may interfere with reactions dependent on folate. The cause of the apparent pyridoxine deficiency is probably even more complex. Increased demand may be a factor but actual interference

with pyridoxine metabolism is likely to be important in many cases (see below)

Response to Folic Acid, Pyridoxine and Crude Liver Extract

It is now widely known that some patients suffering from sideroblastic anaemia respond to folic acid therapy. This was so in approximately one third of our cases of either the primary acquired or hereditary type. The response was, however, generally small and in no case was a normal haemoglobin level achieved. Responses when present were, however, associated with reversal of the marrow picture from megaloblastic to normoblastic.

Some of our patients with hereditary or primary acquired sideroblastic anaemia have shown further responses to pyridoxine, sometimes to small doses, e.g. 0.5 to 1.0 mg per day. Other patients have seemed to need relatively enormous doses. The rise in haemoglobin is often limited and in only a few cases has a normal level been reached and even in these patients minor abnormalities have persisted in the peripheral blood and bone marrow. Two of our patients who were receiving folic acid and pyridoxine, showed some response to crude liver extracts [presumed to contain the haemopoietic factor of Horrigan, Whittington, Weisman and Harris (23)].

Secondary Acquired Sideroblastic Anaemia

In Table IV is shown the wide range of disorders in which ring sideroblasts

in the marrow have been encountered. In some of these patients the role of the sideroblastic change in relation to the patients' anaemia seems to have been a minor one, in others it has probably been a major cause of their anaemia.

The cause of the sideroblastic change in these cases is obscure. Evidence is already available that lead can interfere with haem synthesis at various points. Presumably the anti-tuberculosis drugs act by interfering with pyridoxine metabolism, but at what stage derangement of pyridoxine metabolism interferes with the synthesis of haemoglobin is uncertain (see below). The different anti-tuberculosis drugs differ markedly in their "sideroblastic anaemia producing" effect. Drugs like cycloserine and pyrazinamide appear to produce sideroblastic anaemia far more readily in man and experimental animals than does INH. However, recent studies indicate that abnormalities qualitatively similar to those seen in sideroblastic anaemia but of lesser intensity occur in the bone marrows of a very large proportion of patients receiving INH (33).

The association of leukaemia with sideroblastic change in the marrow is of special interest and has received a good deal of attention (11). In our experience ring sideroblasts are not commonly seen in leukaemia, occasionally, however, a few are present. They seem to be relatively more frequent in myelosclerosis. Exceptionally, in our experience, many are present in both leukaemia and myelosclerosis and in

rare instances the distinction between chronic Di Guglielmo's disease (with sideroblastic change) and primary acquired sideroblastic anaemia may be difficult. But we believe that the majority of patients with acquired sideroblastic anaemia certainly do not have leukaemia. The transformation of the former to the latter may sometimes occur but we do not believe that this often happens. In our opinion there seems no reason why sideroblastic change to a minor or major degree should not occur from time to time in leukaemia as one aspect of the alteration in marrow cell metabolism consequent on leukaemic change.

Pyridoxine Responsive Anaemia

There is now a large literature on the anaemias responsive to pyridoxine and the subject has recently been fully reviewed (22). In the present authors' view these anaemias do not need to be placed in a separate category; they are sideroblastic anaemias of hereditary or acquired types. Although many of these patients respond to treatment with pyridoxine there may be no clinical or laboratory evidence of pyridoxine deficiency.

Some patients have however been recorded in whom the biochemical tests are indicative of pyridoxine deficiency and who respond almost completely to relatively small doses of pyridoxine e.g. 1 mg. daily by mouth (29), 5 mg. daily by mouth (13). In these patients the condition rapidly relapses if treatment is stopped. The

patient studied by McGibbon and Mollin (29) took a very poor diet but as some hypochromia and a few ring sideroblasts persisted even after very large doses of pyridoxine it is unlikely that the metabolic basis for the disorder, even in these patients is uncomplicated pyridoxine deficiency. This is supported by the fact that sideroblastic change is rarely seen in patients with other evidence of nutritional deficiency or in patients with the intestinal malabsorption syndrome. This may be because such patients are usually iron deficient (12). But this is unlikely to be the whole explanation for ring sideroblasts rarely develop when the iron deficiency is corrected.

On the other hand in some secondary sideroblastic anaemias it seems likely that pyridoxine deficiency alone or probably more effectively when associated with folic acid deficiency can produce sideroblastic change. In some of these patients hypochromia and ring sideroblasts disappear when the patients are treated with pyridoxine and folic acid. In other cases these abnormalities disappear with remission of the associated disease.

It seems likely therefore that in certain secondary cases pyridoxine deficiency can by itself or when associated with folic acid deficiency result in a sideroblastic anaemia which is apparently cured when pyridoxine is given as treatment. The results in cases which have followed the use of anti-tuberculosis drugs which are known to act as pyridoxine antago-

nists support this view (26) (31) (28) (36)

It is quite clear that pyridoxine deficiency is *not* the cause of primary acquired sideroblastic anaemia. Instead, an increased requirement for pyridoxine (and folic acid) often appears to be superimposed on an acquired disorder of erythropoiesis — which itself, too, produces ring sideroblasts. Response to pyridoxine and/or folic acid in these cases depends upon the degree and severity of the underlying abnormality and the extent of the overlying deficiency state. Where the response only follows the giving of very large doses of pyridoxine, replenishment of a conditioned deficiency can hardly be the explanation. In these cases it seems more likely that pyridoxine is overcoming partially, by mass action perhaps, a defect in a metabolic process in which a derivative of pyridoxine acts as a co factor.

The Nature of Sideroblastic Anaemia

The disturbance leading to ring sideroblast formation and to sideroblastic anaemia is uncertain. It has been suggested that the primary defect is interference with the synthesis of haem. The synthesis of protoporphyrinogen from glycine and succinate via amino laevulinic acid, porphobilinogen, uroporphyrinogen and coproporphyrinogen and its final linkage with iron to form haem is mediated through a whole system of enzymes and interference or deficiency of some of these enzymes may be the basis of a haem synthesis defect in sideroblastic

anaemia. Pyridoxine is a key substance in this chain of syntheses, for the combination of glycine and succinyl Co-A is brought about by a system requiring pyridoxal or pyridoxal 5 phosphate. Pyridoxal 5 phosphate is also required for the mobilization of iron from mitochondria (7). We have already pointed out that a condition closely resembling naturally occurring sideroblastic anaemia can be produced in man and in experimental animals by drugs that act as pyridoxine antagonists, while patients with naturally occurring sideroblastic anaemia sometimes show evidence of abnormal pyridoxine metabolism.

It should be added that Vavra and Poff (35) could find by *in vitro* studies no direct evidence of a defect in synthesis between amino laevulinic acid and haem in eight patients with sideroblastic anaemia: the defect, however, may occur earlier. Thus lead, which is a well known cause of sideroblastic anaemia, affects haem synthesis prior to amino laevulinic acid formation; it also affects, however, the incorporation of iron into protoporphyrin and probably acts on other stages of porphyrin synthesis, too (16).

A good deal more needs to be known before it is justifiable to come to any firm conclusions as to the pathogenesis of the sideroblastic anaemias. Pyridoxine antagonists act on many enzyme systems and could therefore affect haemoglobin synthesis less directly than we have suggested and there is some evidence which suggests that globin synthesis is ineffec-

tive in some types of sideroblastic anaemia (21)

The role of excess intracellular iron itself is particularly interesting but still needs clarification. It is possible that excess iron present in erythroblasts due to the combined effects of high availability, defective utilization and an increased rate of uptake of iron by the erythrocyte (2) acts as an impediment to haem synthesis. The demonstration of the enormous accumulation of iron in mitochondria in sideroblastic anaemia suggests that mitochondrial activity may be impaired and experimentally Harris, MacGibbon and Mollin (20) showed that drug-induced sideroblastic anaemia in mice and guinea pigs tended to be more severe in animals given an iron supplement. The argument that excess iron cannot be of much importance because haemochromatosis is not accompanied by anaemia is not sound for intra erythroblast iron is not greatly increased in haemochromatosis and ring sideroblasts are not seen. However it has to be admitted that Bishop and Hathaway (3) failed to demonstrate that iron inhibited haem synthesis in an *in vitro* system but the results in the experimental system they used may not be entirely applicable to the unusual conditions found in sideroblastic anaemia.

Summary

The circumstances in which siderocytes and sideroblasts occur in human peripheral blood and bone marrow are briefly reviewed. The different

types of sideroblastic anaemia are described and their pathogenesis discussed.

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Hypochromic Anaemia without Iron Deficiency

By M. C. VERLOOP, P. W. HEILEMAN and K. PUNT

In our contribution to this festive publication commemorating Jan Waldenström's 60th anniversary we should like to discuss some unusual causes of hypochromic anaemia. Sideropenic hypochromic anaemia has been investigated by many authors including Waldenström who as early as 1937 pointed out cutaneous and mucosal changes (21) and described sideropenic dysphagia (22).

Occasionally however one encounters patients with hypochromic anaemia who do not suffer from sideropenia but in fact often show the opposite: haemosiderosis. We intend to present a brief description of some forms of this hypochromic "hyper-sideremic" anaemia.

Introduction

The blood picture in patients with non-sideropenic hypochromic anaemia is usually microcytic. The blood smear is also characterized in many cases by large intensively hypochromic cells known as target cells. Partly due to the presence of these "flit" target cells,

the mean red cell thickness is often diminished while the resistance of the red cells to hypotonic saline solutions in decreasing concentrations is increased.

Differential diagnosis of hypochromic hyper-sideraemic anaemias

When in North West Europe one encounters a hypochromic anaemia not based on iron deficiency three disease pictures must be borne in mind as primary alternatives namely thalassaemia minor α thalassaemia and hereditary siderochrestic anaemia.

In thalassaemia synthesis of normal adult Hb A is diminished because there is a genetically determined disturbance in the production of either the alpha or the beta chains of the globin giving rise to alpha thalassaemia or beta thalassaemia respectively.

Thalassaemia minor (β thalassaemia)

Thalassaemia minor — the heterozygous form of beta thalassaemia can be found in all countries of W. Europe. It is also observed in patients whose ancestors do not seem to come from

Table I *Thalassaemia minor* (β -thalassaemia)

Patients	Sex and Age	Hb g/100 ml	Erythrocytes mill/cu mm	MCH ¹ μ g	MCV ² μ 3	Serum iron		
						Fasting μ g/100 ml	Saturation %	
Normal range				28-32	80-100	80-180	20-60	
Fam	{1	I 18	101	43	24	60	138	31
	2	M 34	106	54	20	63	167	51
	3	M 19	109	48	29	83	142	44
	4	F 61	93	15	22	80	136	44
	5	I 5	98	43	23	84	151	56

	Bone marrow				Haemoglobin				
	Ratio Red White	RES iron	Sideroblasts	Ringed sideroblasts %	Alkaline resistant Hb %	A ₁ %	A ₂ %	H ₂ %	
Normal range	1 (2.5-5)	\pm or +	\pm	0	max 3	max 88	max 3	0	
Fam	{1	1 2	++	+	0	99	94.3	4.8	0
	2	1 1	++	+	0	22	93.1	4.7	0
	3	2 1	+	+	0	14	93.3	4.7	0
	4	1 1	++	+	0	93	98.5	5.7	0
	5					38	91.3	4.9	0

¹ MCH = Mean Corpuscular Haemoglobin² MCV = Mean Corpuscular Volume

countries on the Mediterranean sea (5, 18). The disturbance in haemoglobin formation results in a usually mild to moderately severe hypochromic anaemia, which is often microcytic. Table I presents data on some Dutch families studied by us. The serum iron concentration is normal or slightly increased with usually a normal iron saturation percentage of transferrin. The bone marrow shows hemosiderosis, and a sideroblast count which is slightly above normal. So called ringed sideroblasts — i.e. erythroblasts in which a ring of thick iron granules closely surrounds the nucleus — are sporadically seen, if at all. The condi-

tion is diagnosed on the basis of haemoglobin analysis. The haemoglobin A₂ concentration is as a rule increased and sometimes the haemoglobin F concentration too.

Alpha-thalassaemia (haemoglobin H disease)

The second possibility to be taken into account in the case of "iron saturated" hypochromic anaemia is alpha thalassaemia.

In alpha thalassaemia the production of alpha chains of the globin is retarded, as a result of which the normal haemoglobin synthesis is disturbed and an excess of free beta chains

Table II α Thalassaemia (Haemoglobin H disease)

Patient	Sex and Age	Hb g/100 ml	Erythrocytes mill/cu mm.	MCH: μ g	MCV: μ 3	Serum iron	
						Fasting μ g/100 ml.	Saturation %
Normal range				23-32	80-100	50-150	20-60
1	M 43	10.3	59	18	67	98	37

	Bone Marrow				Haemoglobin				Erythrocytes with BCB ² incl bod ³
	Ratio Red White	RES iron	Sideroblasts	Ringed sideroblasts %	Alkaline resistant Hb %	A ₁ %	A %	H %	
Normal range	1 20-50	\pm or +	\pm	0	max 3	max 98	max 3	0	0
1	2 1	++	+	0	0.0	98.4	0.7	2-4	73

¹ MCH = Mean Corpuscular Haemoglobin

² MCV = Mean Corpuscular Volume

³ BCB = Brilliant Cresyl Blue

can occur. These chains can polymerize to become tetramers and combine with haem to form a new haemoglobin known as haemoglobin H (β_4). Shortly after birth when haemoglobin synthesis is still largely confined to haemoglobin I, an excess of gamma chains can lead in these cases to the formation of Bart's haemoglobin whose globin part consists of a tetramer of gamma chains. The disturbed production of the alpha chains can give rise to a decrease in haemoglobin 1. The alpha chain deficiency also leads to a more or less severe hypochromic anaemia. Variations in the manifestations of α thalassaemia have been described in Weatherall's monograph (26).

In α thalassaemia the amount of haemoglobin H and/or Bart's haemoglobin is usually too small to be readily demonstrable but demonstra-

tion is sometimes possible in these cases by means of a sensitive starch gel electrophoresis technique (15). Moreover a suspicion of α thalassaemia warrants a search for typical red cell inclusions caused by denaturation of labile haemoglobin H. These inclusions can be detected after incubating the erythrocytes with brilliant cresyl blue (19).

Table II presents a number of data on a patient of Dutch extraction with α thalassaemia or haemoglobin H disease. The hypochromic anaemia in α thalassaemia like that in beta thalassaemia is often microcytic. Again the serum iron concentration is normal or slightly increased with usually a normal serum iron saturation percentage. The erythropoietic system in the bone marrow is often hyperplastic, with haemosiderosis in the reticuloendothelial system. As in the

Table I *Thalassaemia minor* (β -thalassaemia)

Patients	Sex and Age	Hb g/100 ml	Erythrocytes mill/cu mm	MCH ¹ μ g	MCV μ 3	Serum iron		
						Fasting μ g/100 ml	Saturation %	
Normal range				28-32	80-100	80-180	20-60	
Fam	1	F 18	10.1	43	24	60	138	37
	2	M 34	10.6	54	20	63	167	51
	3	M 19	10.9	48	29	83	142	33
	4	F 61	9.8	45	22	80	136	44
	5	F 5	9.8	43	23	84	151	36

	Bone marrow				Haemoglobin			
	Ratio Red White	RES iron	Sideroblasts	Ringed sideroblasts %	Alkaline resistant Hb %	A ₁ %	A ₂ %	H %
Normal range	1 (25-50)	\pm or +	\pm	0	max 3	max 98	max 3	0
Fam	1	1 2	++	+	0	99	48	0
	2	1 1	++	+	0	22	93.1	4.7
	3	2 1	+	+	0	14	93.3	4.7
	4	1 1	++	+	0	93	98.5	5.7
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The second possibility to be taken into account in the case of "iron saturated" hypochromic anaemia is alpha thalassaemia.

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Table IV "Thalassaemia like Syndrome"

Table IV - <i>Thalassaemia</i> like syndrome							
Patients	Sex and Age	Hb g/100 ml	Erythrocytes mil/cu mm	MCH ¹ μ g	MCV μ 3	Serum iron	
						Fasting μ g/100 ml	Saturation %
				28-32	80-100	80-150	20-60
Normal range				20	72	186	56
Fam {	1	M 70	109	53	22	80	138
	2	M 30	134	52	23	73	131
	3	M 60	13	56			46

	Bone marrow				Haemoglobin				Erythrocytes with BCB ² incl. bod %
	Ratio Red White	RES iron	Sideroblasts	Ringed sideroblasts %	Alkaline resistant Hb %	A ₁ %	A ₂ %	H %	
Normal range	1 (25-5)	± or +	±	0	max. 3	max 98	max 3	0	0
1 am	1	1 1	++	+	0	13	96.2	2.5	0
	2	1 1	++	+	0	18	95.8	2.4	0
	3					1.3	96.1	2.6	0

¹ MCH = Mean Corpuscular Haemoglobin

² MCV = Mean Corpuscular Volume

³ BCB = Brilliant Cresyl Blue

and iron saturation of transferrin reportedly attains 100 %. In the bone marrow the hyperactive erythropoietic system is usually normoblastic. There is iron storage and the RES of the bone marrow always shows pronounced hemosiderosis. Bone marrow smears stained for iron moreover show typical sideroblasts, the so called ringed sideroblasts in which thick granules of iron are found in the mitochondria immediately surrounding the nucleus (4).

Thalassaemia like syndrome

Sometimes in cases of hypochromic "hypersideraemic" anaemia no signs of the three just described diseases can be detected.

We found an autosomal hereditary hypochromic microcytic anaemia without any sign of alpha or beta thalassaemia in three consecutive generations of the same Dutch family. The anaemia was also found in a female member. These facts ruled out a hereditary sex linked sideroachrestic anaemia. This syndrome is to be discussed in detail elsewhere (7).

Table IV presents data on some members of this family. This hypochromic anaemia again entails a normal to slightly increased serum iron concentration and bone marrow hemosiderosis. Ringed sideroblasts are absent. An exhaustive electrophoretic study up till now failed to disclose an abnormal haemoglobin and there was

Table III *Hereditary Sideroachrestic Anaemia*

Patients	Sex and Age	Hb g/100 ml	Erythrocytes mil/cu mm	MCH ¹ μ g	MCV ² μ 3
Normal range				28-32	80-100
Fam {	1 M 39	10.6	5.3	20	75
	2 M 38	9.0	4.4	20	70
	3 M 36	12.0	5.6	21	67
	4 M 28	9.9	4.5	22	71
Fam {	5 M 54	7.5	5.0	15	52
	6 M 57	11.4	5.3	21	74

	Serum iron		Bone Marrow			
	Fasting μ g/100 ml	Saturation %	Ratio Red White	RES iron	Sideroblasts	Ringed sideroblasts %
Normal range	80-160	20-60	1 (2.5-3)	\pm or +	\pm	0
Fam {	1 236	90	1 1	+++	+++	48
	2 174	49				
	3 124	33				
	4 212	71				
Fam {	5 262	80	1 1/2	+++	++	38
	6 141	36	1 1	+++	++	20

¹ MCH = Mean Corpuscular Haemoglobin² MCV = Mean Corpuscular Volume

lassaemia minor the sideroblast count is increased, but so called ringed sideroblasts are absent.

This patient is the first of Dutch extraction to be described with this condition. A few other authors (3, 12, 22, 29) have likewise described alpha thalassaemia in patients of North West European origin.

Hereditary sideroachrestic anaemia

The third alternative of hypochromic hypersideraemic anaemia is that which occurs as a familial condition in males. The condition is probably subject to a chromosomal transmission and is

known as hereditary sideroachrestic anaemia. It was first described by Cooley (7) and subsequently by many other investigators (13, 17, 20, 27). No abnormal haemoglobin is found in these patients and there are no changes in the amount of haemoglobin A₂ or haemoglobin F.

Table III presents a number of data on a few patients with this condition whom we examined. As in the case of thalassaemia minor and in haemoglobin H disease there is a hypochromic often microcytic anaemia which can range from mild to severe. The serum iron concentration is often increased.

without any iron deficiency in the organism (11 16)

To summarize it is of importance to consider the above described diseases as possibilities in the case of hypochromic anaemia refractory to iron medication and to bear in mind that protracted iron medication may harm these patients who may well show a favourable response to some other form of treatment

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Table V Comparison of some data on the patients listed in Tables I to IV

	Presence of "Ringed Siderobl" Bone marrow	Increase of Hb A ₂	Increase of Hb F	Presence of Hb II	Erythrocytes with B.C.B. ¹ inclusion bodies
1 Thalassemia minor (β thalassaemia)	no	yes	often	no	no
2 α Thalassemia (Hb H disease)	no	no	no	yes	yes
3 Hereditary sidero- achrestic anaemia	yes	no	no	no	no
4 "Thalassemia like syndrome"	no	no	no	no	no

¹ B.C.B. = Brilliant Cresyl Blue

no increase in haemoglobin A₂ or haemoglobin F

This syndrome most closely resembles heterozygous thalassemia. It is remarkable, however, that none of the patients examined (Table IV) showed increased haemoglobin A₂ or haemoglobin F values. Three siblings with the same form of anemia too, presented no clue indicating α or β thalassemia.

A number of authors (1, 2, 6, 10) have reported on patients with hypochromic anemia and a normal serum iron concentration, in whom neither haemoglobin A₂ nor haemoglobin F was increased. But these patients belonged to families with β thalassemia in which several other members did have an increase of haemoglobin A₂ or haemoglobin F.

For the time being, we refrain from classifying the family we described, preferring the designation "thalassaemia like syndrome".

Table V presents data on the differences between the four disease pictures discussed.

Other non-sideropenic hypochromic anaemias

In exceptional cases, hypochromic anemia not caused by iron deficiency can have yet other causes, some of which we intend to mention without discussing them in detail.

Pribilla (19) described a hereditary hypochromic anemia in which a pathological haemoglobin fraction was found now known as haemoglobin Köln.

There are a few reports on hypochromic anemia ascribed to absolute pyridoxine deficiency (8, 23).

Shahidi and Diamond (21) described a hypochromic anemia with an increased serum iron concentration with hemosiderosis in the liver and with no stainable iron in the bone marrow.

In the case of congenital atransferrinaemia as described by Heilmeyer et al. (14), hypochromic anemia also occurs.

Finally, chronic infections can in the long run lead to hypochromic anemia.

aplastic anemia group had decreased circulating reticulocytes and a marked reduction of red cell precursors in aspirated marrow

Internal distribution of iron was studied after the intravenous administration of transferrin bound iron (series I) and after the oral ingestion of radioiron (series II). In the intravenous studies 5 to 40 μC Fe^{59} in the form of iron citrate was incubated with normal plasma which had a binding capacity of more than twice the content of iron added. When iron was given by mouth 20 to 100 μC were employed depending on the amount of absorption anticipated. The form and amount of iron fed is indicated in the individual experiments (Table III). In some individuals both oral and parenteral studies were carried out in order to compare the distribution from the two routes with sufficient time between the two studies to prevent counting interference. Ten to 14 days after the administration of radioiron the amount incorporated into the circulating red cell mass was determined from the formula

$$\frac{\text{counts/ml blood} \times \text{blood volume}}{\text{counts injected}}$$

The blood volume was assumed to be 60 ml/kg of body weight. Plasma iron turnover was calculated according to the formula of Huff *et al.* (5) but modified so as to express turnover per 100 ml whole blood (6) according to the simplified formula

$$\text{Plasma iron turnover (mg/day 100 ml whole blood)} = \frac{\text{Plasma Iron } (\mu\text{g}/100 \text{ ml})}{1/2 \text{ (minutes)}} \times (100 \text{ hematocrit})$$

In vivo counts were recorded over the sternum at the level of the fourth rib and over the center of liver dullness as determined by the percussion in the right anterior axillary line. These counts were carried out at intervals over a period of 14 days after injection. Results were expressed as a simple ratio of counts over the liver divided by counts over the sternum. It was found that at least 6 days were required in the normal subject for stabilization of the liver/sternal ratio. In subjects with direct parenchymal uptake of radioiron from the plasma an early equilibrium was obtained but in patients with ineffective erythropoiesis it was found advisable to wait at least 10 days. Data illustrative of this are shown in Table I.

In vitro counting of blood samples was carried out in a shielded sodium iodide crystal vial counter with a background of approximately 100 c/min. *In vivo* counting was carried out with a 2 inch sodium iodide crystal shielded with 2 inches of lead and collimated 2 inches with a 1 inch aperture. Plasma iron determinations were made by the method of Bothwell and Mollin (7) and iron binding capacity by the method of Zak (8).

Results

The ferrokinetic measurements on six normal subjects given plasma bound radioiron intravenously are shown in Table II. Liver/sternum ratios averaged

A Study of Internal Distribution of Iron in Man

By FAZLE HOSAIN and CLEMENT A. FINCH

The movement of radioiron within the body of man may be followed by *in vivo* counting (1, 2, 3). Such observations provide a means of detecting abnormalities in red cell kinetics and in iron metabolism. Three major pathways of tissue distribution of transferrin bound iron may be illustrated by surface counting (Fig. 1). The first and usually the predominant tissue receptor is the erythroid marrow which incorporates radioiron into new erythrocytes that shortly are released to the blood. The second also involves the primary uptake of radioiron by the marrow, but with subsequent phagocytosis of the newly formed red cells in various parts of the reticuloendothelial system (ineffective erythropoiesis and hemolytic anemias). The third pattern consists of direct uptake of transferrin bound radioiron by parenchymal cells of the liver. It is the concern of this report to establish standards for the practical evaluation of these pathways in man following the intravenous or oral administration of radioiron.

Materials and Methods

The individuals studied were divided into the following categories: 1) normal, 2) iron deficiency, 3) idiopathic hemochromatosis, 4) ineffective erythropoiesis, and 5) aplastic anemia. Normal subjects had hematocrit values of between 40 and 50 per cent and plasma iron levels of 70 to 130 $\mu\text{g}/100$ ml plasma. The diagnosis among subjects in the iron deficiency group was established by the presence of a hypochromic microcytic anemia and a transferrin saturation below 16 per cent (4). The criteria in the idiopathic hemochromatosis group included a near saturation of transferrin, the presence of excessive iron deposits in the parenchymal cells of the liver as determined by biopsy, and no specific cause of iron overload. Patients with ineffective erythropoiesis showed an increase in red cell precursors in the aspirated marrow but normal or depressed circulating reticulocytes. Their plasma iron turnover was increased but red cell utilization of radioiron was depressed. All patients in the

Table III *Distribution of Radioiron After Oral Injection*

Name	Condition	1 CV	SeFe	% Sat	Nature of Dose	Absorption	L/S
Bo	Normal	41	100	32	trans Fe \bar{c} Meal	11.0	1.13
Bo		43	130	39	"	6.1	1.22
Ro		49	106	38	1 mg Fe ascorbate	4.3	1.28
Ha		44	108	33	"	12.3	1.30
Be		45	71	28	20 mg Fe ascorbate	2.9	1.33
Ch	Iron Deficiency	46	74	27	100 mg Fe ascorbate	0.5	1.18
Ni		47	81	24	"	9.1	1.23
Mc		43	97	27	100 mg Fe estrate	1.7	1.24
Ta		36	97	23	"	2.1	1.00
An		27	33	8	5 mg Fe ascorbate	28.9	0.63
Ar	Idiopathic Hemo- chromatosis	27	18	4	"	59.7	0.73
We		45	137	47	3 mg \bar{c} Std Meal	74.0	0.65
Tr		41	237	97	"	4.3	0.48
Lo		44	202	98	"	30.6	2.74
in		41	241	100	"	30.5	2.00
Wa	" "	43	246	95	5 mg Fe ascorbate	19.4	1.61
Na		41	288	97	"	25.8	1.44
Ar		41	298	94	20 mg Fe ascorbate	38.0	1.13
Bo		38	328	83	20 mg Fe ascorbate	58.1	0.73
Ch		42	242	95	"	23.2	2.40
Ar	Thalassemia	45	173	64	100 mg Fe ascorbate	7	1.52
Bo		43	257	82	"	7	3.53
Ro		22.5	1.5	76	1 mg Fe \bar{c} Meal	4.8	1.30
Ha	Infractory Anemia	37	266	92	20 mg ascorbate	0.7	0.79

1 to 10 mg iron orally absorption is judged by the amount of radioactivity appearing in the red cells at two weeks was between 0.5 and 12.5 per cent. Liver:sternal ratios varied from 1.13 to 1.33. In two iron deficient subjects there was 1.20 per cent and 60 per cent incorporation of the administered radioiron into red cells and the liver:sternal ratios were 0.63 and 0.73. In four patients with idiopathic hemochromatosis given 3 to 5 mg iron orally liver:sternal ratios varied from 0.63 to 0.43. The subject with the low ratio had been depleted of iron by

phlebotomy and had a normal serum iron. The highest value clearly fell outside of the ranges obtained after intravenous injection of transferrin bound iron in patients with idiopathic hemochromatosis. One of these patients (Bo) had symptoms of weakness, nausea and sweating following 100 mg iron as ferrous sulfate and the liver:sternal ratio was 3.53 at two weeks. Similar symptoms were seen in a normal subject who had been given 100 mg iron as ferrous ascorbate and was placed in a prone position on his left side for two hours. His plasma iron

Table I Liver/Sternal Ratios During Two Weeks

Normal subjects with intravenous Fe^{59}		Thalassemia case with intravenous Fe^{59}		Hemochromatosis patient with oral Fe^{59}	
Days	Ratio	Days	Ratio	Days	Ratio
0	1.01	6	1.35	7	6.42
8	1.14	8	1.47	10	7.90
10	0.96	10	1.70	12	6.51
13	0.93	13	1.73	14	5.08
				17	6.93

ed 1.0, and ranged from 0.85 to 1.13. Between 80 and 85 per cent of the injected radioiron was found in the circulating red cells at two weeks. Four patients with iron deficiency anemia were studied. Ninety eight per cent of the injected activity localized in the circulating red cells, and the liver/sternal ratios averaged 0.78 with a range from 0.74 to 0.85. Virtually all injected radioiron was localized in the circulating erythrocyte mass. Six patients with idiopathic hemochromatosis in various phases of phlebotomy therapy were studied. One of these patients (Ar) had been iron depleted during the preceding year through phlebotomies and despite his high serum iron, had very little excess iron in tissues. His per cent utilization was 78, and his ratio was 1.02. The other patients all had some degree of iron overload when studied and their liver/sternal ratios ranged from 1.34 to 2.62. Their red cell utilization was 49 to 76 per cent. In three patients with ineffective erythropoiesis whose red cell utilization was only 20 to 31 per cent ratios of 1.18, 2.06 and 2.68 were ob-

Table II Iron Turnover and Distribution After Intravenous Injection of Plasma-Bound Radioiron

	Uret	Sple	% Sat	% Util	1:1	1:5 ratio
Group I — Normal Controls						
Es	49	99	34	83	0.63	0.93
Ha	45	—	40	80	0.79	0.97
Gr	47	93	33	83	0.63	1.13
At	49	123	41	84	0.67	1.03
Pe	46	130	52	61	0.67	0.83
He	44	112	48	81	0.73	1.00
Group II — Iron Deficiency						
Dr	50	27	7	100	0.68	0.83
Mi	69	49	13	100	0.84	0.74
Jo	26	43	11	94	1.39	0.68
Fr	26	29	8	100	1.43	0.81
Group III — Idiopathic Hemochromatosis						
Ch	42	162	81	67	0.94	1.90
Wa	37	238	96	73	1.23	1.61
We	38	223	100	76	1.21	1.34
Ar	45	237	83	78	1.02	1.07
Tr	44	192	83	76	0.99	1.18
Po	48	202	79	49	0.81	2.62
Group IV — Ineffective Erythropoiesis						
Wi	30	180	50	20	4.67	2.68
Wo	23	173	92	22	6.87	1.18*
Ba	32	159	87	31	1.97	2.06
Group V — Aplastic Anemia						
Lu	32	158	57	8	0.34	4.23
Si	19	207	93	0	0.40	11.01
Di	22	119	100	<1	0.28	2.90

tained while in three patients with aplastic anemia with over 90 per cent of iron in the tissues ratios of 2.9, 4.2 and 11.0 were observed.

In the second series of studies, radioiron was administered by mouth and results of this are shown in Table III. In normal individuals given from

Table III *Distribution of Radiiron After Oral Injection*

Name	Condition	ICV	SeFe	% Sat	Nature of Dose	Absorption	L/S
Bo	Normal	41	100	32	trans Fe \bar{c} Meal	11.0	1.13
Bo		43	130	39	"	6.1	1.22
Bo		41	106	38	1 m _g Fe ascorbate	4.5	1.28
Ha		44	108	33		12.3	1.35
Be		45	71	28	20 mg Fe ascorbate	2.9	1.33
Ca	Normal	46	74	27	100 mg Fe ascorbate	0.5	1.18
Ni		47	81	24		9.1	1.23
Me		43	97	27	100 mg Fe citrate	1.7	1.24
Ta		40	97	29		2.1	1.06
An		43	97	27		1.7	1.24
An	Iron Deficiency	43	33	8	5 mg Fe ascorbate	28.0	0.63
Ca		27	18	4		50.7	0.43
Ar	Idiopathic Hemo- chromatosis	45	137	47	3 mg \bar{c} Std Meal	74.0	0.65
We		41	237	97		4.3	0.48
Tr	"	44	202	96		30.6	2.74
Do		41	241	100		30.5	2.00
Lo		43	246	95	5 mg Fe ascorbate	19.4	1.61
Wa		41	268	97		25.8	1.44
Ar		41	298	94	20 mg Fe ascorbate	38.0	1.13
Bo	Idiopathic Hemo- chromatosis	38	128	83	20 mg Fe ascorbate	58.1	0.73
Ch		42	242	95	"	23.2	2.40
Ar	"	45	173	64	100 mg Fe ascorbate	?	1.52
Bo		43	257	82		?	3.53
Bo	Thalassemia	22	153	76	1 mg Fe \bar{c} Meal	4.8	1.30
Ha		37	265	92	20 mg ascorbate	0.7	0.70

1 to 10 mg iron orally absorption as judged by the amount of radioactivity appearing in the red cells at two weeks was between 0.5 and 12.5 per cent. Liver sternal ratios varied from 1.13 to 1.33. In two iron deficient subjects there was 1.20 per cent and 60 per cent incorporation of the administered radiiron into red cells and the liver sternal ratios were 0.63 and 0.73. In four patients with idiopathic hemo-
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rose to 320 $\mu\text{g}/100\text{ ml}$, and transferrin became saturated. His liver sternal ratio at two weeks was 2.10. Another patient (H7) with a hyperplastic erythroid marrow, ineffective erythropoiesis, and a saturated transferrin, had a ratio of 6.8 after radioiron administration.

Discussion

In these studies, the internal distribution of iron has been evaluated by surface counting over sternum and liver. The former reflects activity in blood and to some extent, in bone marrow, the latter principally reflects the activity localized in parenchymal and Kupffer cells of the liver, though some contribution was also made by blood and overlying rib marrow. The ratio is taken as an index of liver localization of radioiron, although the quantitative limitations on this interpretation are obvious. The determination of the amount of activity localized in the circulating red cells is of additional help in interpreting the ratio.

The kinetics of transferrin bound iron have been described in the normal subject (3). It is known that 80 per cent of radioiron disappears from plasma to reappear in the circulating red cell mass. The exact whereabouts of the remaining 20 per cent is not known. Surface counting can do no more than provide information of the relative concentration of iron in one area as compared with another. In the normal subject there is little evidence of organ localization at a period of two weeks after injection of radioiron

when the distribution is reasonably stabilized. Activity over sternum and liver are almost identical. However, when comparison with the iron deficient subject who has virtually no deposition of radioiron in tissues is made, it is apparent that there is some increase in the liver as compared with the sternal area. On the other hand, greater accumulations of radioactivity occur in iron overload states and in certain anemias where there is excessive breakdown of red cells, whether it is defined as ineffective erythropoiesis or hemolysis of young circulating cells.

It has been previously pointed out that liver localization may represent either direct storage of iron from transferrin in the liver parenchyma or the secondary uptake of iron from defective red cells by the reticuloendothelial tissue of the liver (9). Direct parenchymal uptake occurs in iron overload conditions and in aplastic anemia, whereas reticuloendothelial uptake occurs with breakdown of newly formed red cells. The different time relationships of these two pathways have already been illustrated (Fig. 1) and are not a consideration in the studies reported here. It is of interest here to compare the amount of tissue storage as determined by the per cent utilization of radioiron with the liver sternal ratios (Fig. 2). As might be expected with increased storage the ratio increases. However in those conditions (ineffective erythropoiesis and hemolytic anemia) where tissue storage occurs throughout the entire reticuloendothelial

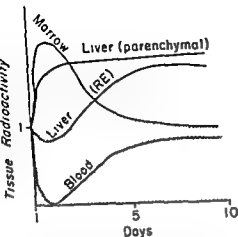


Fig 1 Radioactive iron distribution following intravenous injection of transferrin bound radioiron. Activity immediately after injection is taken as 1. Organ counts are determined by *in vivo* counting. The two liver curves indicate uptake directly from transferrin (parenchymal) and from non viable red cells released from the marrow (RE).

system is compared to parenchymal uptake alone there is proportionately less increase in the liver activity. Another relationship examined was that between the serum iron level or per cent saturation of transferrin and the amount of liver iron deposition (Fig 4). It has been postulated (9) that uptake by liver parenchyma might be dependent on a relation between the serum iron level and the transport protein. With low plasma iron and low per cent saturation as in iron deficiency or infection nearly all radio iron is localized in the red cell mass. When liver uptake is increased a high plasma iron and high transferrin saturation is present. However, as illustrated by patient Ar who had a nor-

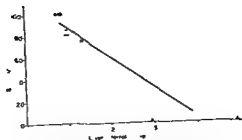


Fig 2 Relationship between body iron distributions (liver/spleen ratio) and amount of iron found in circulating red cells at 2 weeks after intravenous injection of transferrin bound Fe^{59} . The open circles are patients with iron deficiency, closed circles represent normal subjects, squares are idiopathic hemochromatosis, triangles are aplastic anemia and crosses are ineffective erythropoiesis.

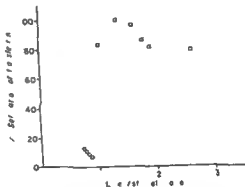


Fig 3 Relationship between body iron distribution and % saturation of transferrin (plasma iron divided by total iron binding capacity). Symbols are the same as in Fig 2.

mal liver/spleen ratio despite a plasma iron of $257 \mu\text{g}/100 \text{ ml}$ and a saturation of 83 per cent it appears to be a permissive rather than an obligatory requirement.

With these considerations concerning internal iron distribution in mind the distribution of iron administered by mouth may be examined. In nor-

mal subjects, in patients with iron deficiency anemia and in most patients with idiopathic hemochromatosis, there was no great difference in distribution between oral and intravenous administration. This confirms the validity of the double isotope method of measuring iron absorption, where a second isotope of iron was used to correct for the distribution of transferrin bound iron to other than the circulating red cells (10). However, in those instances where a high degree of saturation of transferrin was found, there was sometimes a far greater liver accumulation than would have been anticipated from the intravenous studies. Its occurrence in one patient with hemochromatosis, given only 3 mg iron salt along with a test meal suggested that it may occur with normal iron intake. These studies are confirmatory of the work of Wheby *et al* who demonstrated in animals (11) and later in man (12) that if iron is infused to the point of saturation of transferrin iron absorbed from the gastrointestinal tract will be deposited directly in the liver rather than being distributed by the transferrin system. Iron so absorbed will not be measured by blood sampling techniques and could account for some of the discrepancies in the literature of absorptive measurement between stool and red cell methods. It also may explain the lower normal values reported with blood sampling techniques in idiopathic hemochromatosis in its advanced stages. Such abnormality in distributions may be detected either by moni-

toring over the liver and sternum as reported here or by total body counting (13).

Summary

Surface monitoring of the liver and splenic areas has been employed as a means of detecting the degree of hepatic storage of radioiron. In the present study, data were obtained on subjects with varying disorders affecting tissue distribution of radioiron after intravenous and oral administration. In patients with increased parenchymal deposition the liver:sternal ratio was increased in general proportion to the amount of tissue deposition. A high percent saturation of transferrin or high serum iron was usually but not invariably associated with an increased deposition of iron in the liver parenchyma. Less pronounced liver iron deposition occurred as a part of red cell breakdown within the entire reticuloendothelial system. In most subjects distribution after oral iron administration was similar to that after intravenous iron. However in some patients with iron overload and a saturated blood transferrin there was evidence of direct hepatic uptake of iron. It is concluded that surface monitoring may be employed to determine the general distribution of absorbed iron and whether or not this iron has been distributed by the normal plasma transport mechanism.

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Iron nutrition and iron deficiency

By LARS GARBY

The relation between iron nutrition and iron deficiency has long been a matter of considerable discussion. The pioneering study of Waldenström (1946) is no doubt one of the most important precipitating events in this debate. Waldenström presented evidence, based on clinical and hematological data, that no less than 25 % of women in several communities in the County of Uppland in Sweden were iron deficient. If iron deficiency is defined on the basis of hemoglobin values less than 11 to 12 g/100 ml, large survey studies (Trench 1955, Fry 1961, Kilpatrick 1961, Anttila 1962) indicate that iron deficiency is still quite common in many parts of the Western world. Most investigators seem to believe that the condition so defined can, in the great majority of instances, be prevented by a fairly moderate increase in the food iron intake.

Reluctance in accepting definitions of iron deficiency on the basis of hemoglobin values alone have prompted considerations of data concerning the iron balance, i.e. intake, retention, ab-

sorption and excretion. These data are usually interpreted to show that the iron balance is indeed critical (see for instance Moore 1964 and Bothwell and Finch 1962). Hallberg (1964) added new information on the loss of iron in menstrual blood and by using conventional estimates of iron balance data arrived at the conclusion that more than 25 % of menstruating women in Gothenburg, Sweden are in a status of negative iron balance on an intake of 15 mg of iron per day. Since there is good evidence (Söderberg 1958 and 1959, Sterky 1962, see also Blom 1965) that a considerable proportion of women in Sweden have a lower daily intake, these figures predict a very high incidence of iron deficiency.

In the present paper some of the basic data of iron balance are critically discussed. Reasons are given for the necessity of reevaluating some of these data. The results appear to indicate that there is little evidence to suggest that more than a very small proportion of individuals are in a status of negative iron balance when

they are on a daily intake of 10—13 mg of iron

Definition of terms

A scrutiny of the literature reveals the fact that terms like absorption, excretion and retention have been used in a loose way. It will be shown below that a rigorous definition of terms is not only of academic interest. The terms used in the following are defined as follows:

Retention $R = \text{intake } I - \text{output } O$. This is the equation of material balance resulting from two rate processes I and O of which I must be assumed to induce perturbations in the system. Experimentally there are only average values over specified time periods at constant intake are meaningful.

Output O the sum of fecal output (O_f) and urinary output (O_u) and menstrual blood flow output (O_m). Other sources of output may be significant under special circumstances i.e. sweat and dermal cells.

Fecal output O_f = output of unabsorbed iron or iron derived directly from dietary sources (O_{fd}) plus iron of body origin (O_{fb}). The exact internal interpretation of the latter quantity is the output of iron in the faeces at zero intake with the assumption that O_{fb} is independent of I . This assumption is probably true. Most of the flow O_{fb} consists of the iron and erythrocytes but it may be false. The output of the flow is made up of desquamated cells and erythrocytes shed in iron absorption.

Excretion $E = I - O_{fd}$. The experimental measurement of the excretion is so defined as $E = I - O_{fd}$ with the assumption concerning O_{fb} independent.

Tracer absorption is defined experimentally as the difference between the amount of radioactivity administered orally and that recovered in the faeces during a specified period of time on its relation to the actual intake of iron. The actual output is defined by excretion as performed at a steady state and their interpretation is dependent of the details of the mechanism

by which iron is absorbed. The tracer absorption A on the other hand is defined by experiments in which the details of the mechanisms are essential for a correct interpretation of A in terms of A . The generally accepted model for iron absorption at the cellular level proposed by Crosby (1963) and supported by experimental data by his group (Weintraub, Conrad and Crosby 1964; Conrad, Weintraub and Crosby 1964 and Wiley, Jones and Crosby 1964) visualizes an iron pool in the absorbing epithelial cells situated between the luminal iron and the body proper (plasma). Furthermore, the theory includes the notion of a movement of these cells along the crypts and a final desquamation of the cells at the end of the life span. Depending on the assumptions made concerning the magnitude of the intracellular iron pool in comparison with the net transfer and the physical nature of the flow of iron across the two cellular boundaries different situations arise with respect to the interpretation of the observed quantity A . Clearly A may be smaller, equal to or larger than A . Perhaps the most realistic assumptions are 1) that the iron pool is not negligible with respect to the net transfer 2) that the iron flows across the boundaries are one way flows and 3) that some iron is still present in the cells when they are desquamated. In this case it will be smaller than A .

The data

Carefully controlled chemical balance studies have been performed by Johnston and coworkers (Johnston, Frenchman and Boroughs 1948; Schlaphoff and Johnston 1949; McMillan and Johnston 1951 and Ingalls and Johnston 1951). In these studies the output of faecal iron was recorded at fixed iron intake levels during time periods usually four weeks that ensure a reasonable steady state. In terms of the definitions given above

the relation between the observed quantities O_t and I is

$$O_t = I - A + O_{fb}$$

so that only the difference $A - O_{fb}$ can be evaluated. In all but two of 39 recorded periods of measurements of four weeks in 17 women, this difference was found to be positive at intake levels between 0 and 13 mg per day. At an intake level between 10 and 13 mg per day the mean value from 21 periods in 12 women was 1.6 mg per day (range 0.4–2.7 mg per day). The larger part of the variation is undoubtedly due to experimental error.

Although the difference $A - O_{fb}$ is all that is needed to discuss the retention of iron, we shall proceed to analyze its two components A and O_{fb} in order to inquire into the possible connection between them and the data obtained by studies with radioactive iron. The value of O_{fb} at zero intake was estimated by Ingalls and Johnston (1954) by extrapolating the regression of O_t on I to zero intake, a value of 0.2 mg per day was obtained. If it is assumed that O_{fb} is independent of the intake value the absorption A is then 1.8 mg per day at these intake levels or 14–18 %. As pointed out above however, the assumption of independency of O_{fb} on I may not be realistic. Therefore, the average absorption value of 1.8 mg is most probably a minimum value for example if the faecal output of body iron is instead 0.4 mg per day the average absorption is 2.0 mg or 15–20 %.

Tracer absorption studies have been

carried out by several groups of investigators. The results have been summarized by Moore (1964) who quotes an over all average figure of 6.5 % from 133 experiments on normal subjects receiving labelled food containing between 1 and 17 mg of iron. The variation between individuals is quite large in such studies and its source largely unknown. However it appears highly probable that the difference between the chemical balance absorption figure of 14–18 % and the tracer absorption figure of 6.5 % is significant. There are two possible explanations for this rather remarkable difference. Firstly, as was pointed out above, there is no real theoretical basis for assuming that the tracer absorption figure should be equivalent to the absorption figure derived from a balance study. In fact reasonable assumptions concerning the details of the mechanisms of iron absorption make it probable that the tracer absorption figures are underestimations of the true absorption A . Another explanation for the difference may be found in the fact that the figures from the balance data were obtained from young menstruating female individuals whereas most of the tracer studies have been performed on men. This aspect will be discussed below.

The urinary output of iron amounts to 0.1–0.2 mg per day (Johnston and McMillin 1952; Hallberg 1964). The menstrual blood flow output varies considerably between individuals. Hallberg, Hogdahl, Nilsson and Rybo (see Hallberg 1964) have given the

following figures based upon direct measurements in 450 women who were randomly selected in Gothenburg Sweden 30% lost up to 0.6 mg per day 75% lost up to 0.9 mg per day and 90% lost up to 1.4 mg per day. These figures are in good agreement with those given by Lrenchman and Johnston (1949) who found 184 cycles and 270 menstrual periods in the literature where direct measurements had been carried out.

The sum of the terms O_n , O_b and O_m is thus 0.1 mg per day in the average man and non menstruating female and 1.0 mg per day in the average menstruating female. These figures are slightly lower but entirely compatible with the figures estimated by Finch (1964) on the basis of measurements of the decrease in erythrocyte specific activity after intravenous injection of radioiron and of certain assumptions concerning the mixing properties of body iron. Similar estimates have been made it by using whole body counters to measure the decrease in radioactivity. So far however the published studies have failed to meet the requirements of elementary tracer theory that such data can be used to estimate the iron output if and only if 1) the isotope is given in the natural form i.e. in labelled food 2) the loss of radioiron is followed to completion i.e. the total integral is evaluated and 3) the total body iron content is known.

The balance

It follows from the above calculations that the average menstruating woman

retains about 0.6 mg of iron per day on a daily intake of 10–13 mg of iron in a well balanced diet. This amounts to some 200 mg per year. Although the variation of body iron content with time is not known such a figure is almost certainly not representative for women throughout their life and indicates that many of the individuals studied by Johnston and coworkers were in a period of an unusually large positive retention. Six women were still growing. As judged by haemoglobin, red cell count, serum iron and food history, none of the individuals studied had any signs of decreased iron stores. Normal menstrual blood loss was demonstrated in twelve of the women.

The average man and non menstruating female loses about 0.4 mg of iron per day. An average absorption of 4% on a daily intake of 10 mg would therefore be sufficient to ensure a non negative iron balance.

Some 10% of menstruating women lose more than 1.8 mg per day i.e. more than the average amount absorbed. Thus 10% of menstruating females could be expected to be in a situation of negative iron balance. However this estimate ignores the possibility that the absorption and the faecal output of body iron is dependent on the body iron content when this is within "normal" limits. Since such a dependency has been clearly demonstrated with respect to tracer iron absorption of labelled food when the body iron content is abnormally low it is reasonable to expect that it exists also within the "normal" limits.

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so that only the difference $A - O_{fb}$ can be evaluated. In all but two of 39 recorded periods of measurements of four weeks in 17 women, this difference was found to be positive at intake levels between 6 and 13 mg per day. At an intake level between 10 and 13 mg per day the mean value from 21 periods in 12 women was 1.6 mg per day (range 0.4—2.7 mg per day). The larger part of the variation is undoubtedly due to experimental error.

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following figures based upon direct measurements in 400 women who were randomly selected in Gothenburg, Sweden: 50% lost up to 0.6 mg per day, 75% lost up to 0.9 mg per day and 90% lost up to 1.4 mg per day. These figures are in good agreement with those given by Frenchman and Johnston (1949) who found 184 cases and 276 menstrual periods in the literature where direct measurements had been carried out.

The sum of the terms O_1 , O_2 and O_3 is thus 0.4 mg per day in the average non-menstruating female and 1.0 mg per day in the average menstruating female. These figures are slightly lower but entirely compatible with the figures estimated by Finch (1964) on the basis of measurements of the decrease in erythrocyte specific activity after intravenous injection of radioiron and of certain assumptions concerning the mixing properties of body iron. Similar estimates have been aimed at by using whole body counters to measure the decrease in radioactivity. So far, however, the published studies have failed to meet the requirements of elementary tracer theory that such data can be used to estimate the iron output if and only if: 1) the isotope is given in the natural form i.e. as labelled food; 2) the loss of radioiron is followed to completion i.e. the total integrated is evaluated; and 3) the total body iron content is known.

The balance

It follows from the above calculations that the average menstruating woman

retains about 0.6 mg of iron per day on a daily intake of 10–13 mg of iron in a well balanced diet. This amounts to some 200 mg per year. Although the variation of body iron content with time is not known, such a figure is almost certainly not representative for women throughout their life and indicates that many of the individuals studied by Johnston and coworkers were in a period of an unusually large positive retention. Six women were still growing. As judged by haemoglobin, red cell count, serum iron and food history, none of the individuals studied had any signs of decreased iron stores. Normal menstrual blood loss was demonstrated in twelve of the women.

The average man and non-menstruating female loses about 0.4 mg of iron per day. An average absorption of 4% on a daily intake of 10 mg would therefore be sufficient to ensure a non-negative iron balance.

Some 10% of menstruating women lose more than 1.8 mg per day, i.e. more than the average amount absorbed. Thus 10% of menstruating females could be expected to be in a situation of negative iron balance. However, this estimate ignores the possibility that the absorption and the faecal output of body iron is dependent on the body iron content when this is within "normal" limits. Since such a dependency has been clearly demonstrated with respect to tracer iron absorption of labelled food when the body iron content is abnormally low, it is reasonable to expect that it exists also within the "normal" limits.

It may therefore be concluded that the available data concerning the iron balance do not support the idea that iron deficiency is likely to develop in menstruating females on a food iron intake of some 10—13 mg per day

Summary

The available data concerning the iron balance, i.e. absorption, excretion and retention, is critically discussed. Reasons are given for the necessity of re-evaluating some of the data.

The results appear to indicate that, contrary to currently expressed ideas there is little evidence to suggest that more than a very small proportion of individuals are in a status of negative iron balance on a daily intake of 10—13 mg of iron.

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Iron Absorption after Partial Gastrectomy

A comparative study on the absorption
from ferrous sulphate and hemoglobin

By LEIF HALLBLING, LEONART SÖLVELL and BLAUG ZEDERFELDT

Iron deficiency anemia is common after partial gastrectomy — it is more common in women than in men and more common after operations of the Billroth II type than after the Billroth I type (1-3).

The main causes of the postgastrectomy iron deficiency anemia discussed are (1) blood loss, (2) reduced intake of food iron and (3) impaired absorption of iron from the food. Gastrointestinal occult blood loss is probably not a main cause of the anemia as the incidence of positive benzidine reactions was the same in anemic and non-anemic patients after partial gastrectomy as in non-gastrectomized peptic ulcer patients (4). Moreover the loss of body radioiron over a period of one year is the same in non-gastrectomized patients (of whom 3 were anemic) as in normals (10). Both in men and in women a lower intake of iron with food was observed in anemic than in non-anemic gastrectomized patients. However a low intake of iron cannot

be the sole explanation of the anemia as in anemic men the average daily intake of iron was 115 mg. This amount should be sufficient to balance the small daily losses of iron in men.

Because of this the main attention has been given to the ability to absorb iron after partial gastrectomy.

The absorption of iron from iron salts seems to be unaffected by gastrectomy (1-9) and it has repeatedly been shown that iron deficiency anemia after partial gastrectomy responds to oral iron therapy (11). A marked reduction of the absorption has been observed in most studies on food iron absorption (2, 4, 10). However in a recent study on food iron absorption measured by adding an inorganic radioiron tracer to a meal the effect of gastrectomy was very slight (7).

The great variation in the absorption of iron between and within individuals makes it difficult to determine if the absorption is affected by the gastrectomy or not in the single indi-

Table 1 Absorption of Iron from Ferrous Sulphate and Hemoglobin in Normals, Blood Donors, and Patients with Iron Deficiency Anemia

	Case	Age	Sex	Hb conc g/100 ml	MCHC (per cent)	Iron Absorption (per cent)		Absorption ratio FeHb/FeSO ₄
						HbFe	FeSO ₄	
Normals	N 1	23	M	15.2	32.3	4.9	5.0	0.98
	N 2	25	M	14.5	33.0	4.6	3.4	1.35
	N 3	23	M	13.7	32.6	2.9	3.0	1.07
	N 4	24	M	13.1	31.2	4.5	3.3	1.33
								Mean 1.17
Blood Donors	BD 1	40	M	15.6	31.8	27.4	60.7	0.45
	BD 2	40	M	15.5	33.0	10.9	35.3	0.31
	BD 3	48	M	14.4	31.3	8.7	48.2	0.18
	BD 4	40	M	13.6	31.6	25.9	62.0	0.41
Iron Def Anemia	A 1	71	F	11.4	30.0	13.5	47.5	0.29
	A 2	39	F	10.5	30.0	14.6	32.1	0.46
	A 3	54	F	9.3	29.1	10.0	40.0	0.25
								Mean 0.34

vidual. When studying the effect of gastrectomy on the absorption of iron salts and of food iron it is thus important to make comparative absorption studies in the same subject. Such comparisons are greatly facilitated by using a double radioiron technique. In the present study a comparison was made of the absorption of iron from ferrous sulphate and from hemoglobin which were labelled with two different radioiron isotopes and administered on alternate days to the same subject. The study included two groups of patients with Billroth I or Billroth II gastrectomy; in these two operations differ significantly in their tendency to develop iron deficiency anemia. A group of non-gastrectomized subjects was also studied in the same way to get a firmer basis for the evaluation of the effect of gastric resection on the absorption of iron.

Methods

A double radioiron technique with the same experimental design as previously described was used (3). Five milligram of iron was given orally every morning for 10 days after an overnight fast. Solutions of ferrous sulphate and solutions of hemoglobin (hemolyzed red cells) labelled with different radioiron isotopes (^{55}Fe and ^{59}Fe) were given on alternate days. Each dose (25 ml) also contained 5 mg ascorbic acid and 10 ml 70 per cent black currant syrup. Two weeks after the last oral dose a blood sample was drawn for determination of ^{55}Fe and ^{59}Fe . The details of the method were the same as previously described.

The ferrous sulphate was labelled with ^{55}Fe and the hemoglobin with ^{59}Fe . The labelled hemoglobin was prepared by giving 500—1000 μC of FeCl_3 (about 15 $\mu\text{C}/\text{g}$ Fe) intravenously to a rabbit. One week later the rabbit was exsanguinated by carotid artery bleeding. The red cells were hemolyzed by freezing and thawing. Each dose of "hemoglobin iron" contained about 4 μC ^{59}Fe and was diluted with non-radioactive frozen and thawed rabbit red cells to obtain 5 mg of elemental iron in each dose.

Table II Absorption of Iron from Ferrous Sulphate and Hemoglobin in Patients with Partial Gastrectomy (Billroth I)

Case	Age	Type of ulcer	Hb conc. g/100 ml	MCHC (per cent)	Iron absorption (per cent)		Absorption ratio (Fe/Hb FeSO ₄)
					HbFe	FeSO ₄	
1	40	D	12.3	31.9	36	3	0.99
2	50	D	12.2	32.3	37	29	1.25
3	45	D	14.7	32.0	60	27	1.05
4	43	D	14.1	32.0	20	21	0.96
5	57	D	13.6	32.4	63	21.0	1.30
6	49	D	13.5	31.0	79	17.4	0.45
7	40	D	12.3	31.1	27	26	1.02

(Gastric D=Duodenal)

Table III Absorption of Iron from Ferrous Sulphate and Hemoglobin in Patients with Partial Gastrectomy (Billroth II)

Case	Age	Type of ulcer	Hb conc. g/100 ml	MCHC (per cent)	Iron absorption (per cent)		Absorption ratio (Fe/Hb FeSO ₄)
					HbFe	FeSO ₄	
1	46	D	14.1	29.4	12.5	13.1	0.95
2	44	D	13.5	31.4	16.6	20.5	0.81
3	47	D+G	13.2	28.7	15	14.1	1.12
4	40	D	12.9	29.7	5	3.1	2.43
5	48	D	12.1	28.1	23	27	1.61
6	50	G	11.1	27.8	29	39	1.34

(Gastric D=Duodenal)

Materials

Thirty male gastrectomized patients were included in the study. In all patients a partial gastrectomy was done about 3 years ago for the removal of gastric ulcer. Seven patients were operated according to Billroth I and 3 patients according to Billroth II (see Table II and III). None was anemic at the time of the operation and gastrointestinal function was in accordance with the indication for the operation. In all patients there was no recurrent ulcer, no bleeding and no iron deficiency. The blood loss at the operation was estimated but not estimated retroactively to the 3 patients of blood transfusion during the operation.

Two groups of non-gastrectomized subjects were also studied. One group consisted of 4

healthy male medical students (A) the other one 15 subjects with iron deficiency. The latter group comprised 4 healthy non-anemic male blood donors (B) who had given 68 units of blood during the last 10 years (see Table I) and who had never had any iron supplementation. The group also included 3 patients with iron deficiency anemia (A).

Results

A. Non-gastrectomized subjects

As shown in Table I the absorption of iron from ferrous sulphate and from hemoglobin was about the same in the 4 normal subjects. In the iron deficient

group, there was an increase in the absorption of iron from both compounds but a markedly greater increase in the absorption from ferrous sulphate than from hemoglobin giving an absorption ratio ($\text{FeHb}/\text{FeSO}_4$) of about 0.3

B Partially gastrectomized subjects

1 *Billroth I group* The results from the 7 male patients operated according to Billroth I are given in Table II. In the 4 subjects with a hemoglobin concentration above 14 g/100 ml blood the absorption of iron from ferrous sulphate and from hemoglobin was about the same and of the same magnitude as observed in the normal subjects.

In subjects 5 and 6, who had a hemoglobin concentration below 14 g/100 ml blood there was an increased absorption of iron from ferrous sulphate and possibly a somewhat higher absorption of hemoglobin iron compared to subjects 1—4. Subject 7 showed a low absorption both from ferrous sulphate and from hemoglobin. However this subject differed in one respect from all the others by having evident dumping symptoms each time the sugar containing iron solution were taken.

2 *Billroth II group* The results from the 6 male patients operated according to Billroth II are given in Table III. Judging from the MCHC values, they were all iron deficient and all but one had hemoglobin values below 14 g/100 ml blood. The three subjects (4—6) with the lowest hemoglobin values had a markedly lower ab-

sorption from both ferrous sulphate and hemoglobin than subjects 1—3.

Discussion

It is difficult to study the effect of gastrectomy on the absorption of iron. The difficulty cannot be overcome by studying the same patient before and after the operation as e.g. the size of the iron stores on the two occasions is not known. In the present study, the effect of gastrectomy on the iron absorption was analyzed not only by comparing the iron absorption from ferrous sulphate and hemoglobin in the same patient but also by comparing the absorption ratio (hemoglobin iron/ferrous iron) with the ratio in non gastrectomized subjects — normals and subjects with various degrees of iron deficiency. The interpretation of the results in the gastrectomized patients was greatly facilitated by the following observations: (1) in normal subjects, the absorption of hemoglobin iron and ionized iron was low and about the same; (2) in non gastrectomized iron deficient subjects, the absorption was increased from both types of iron compounds but significantly more from the ionized iron.

In the Billroth II group the markedly increased absorption of hemoglobin iron in subjects 1—3 was probably due to an iron deficiency. In the iron deficient non gastrectomized subjects the absorption of iron from ferrous sulphate was about 3 times higher than from hemoglobin. However in these Billroth II subjects (1—3) the absorption of iron from ferrous

sulphate was increased only up to the same level as the absorption from hemoglobin. Thus the absorption of iron from ferrous sulphate was more impaired than that from hemoglobin. In subjects 4-6 who had the lowest hemoglobin values of the Billroth II group the absorption from both hemoglobin and ferrous sulphate was evidently reduced compared with subjects 1-3. The reduction was most marked for ferrous sulphate giving an absorption ratio ($\text{FeHb}/\text{FeSO}_4$) much above 1 against an expected ratio of about 0.3 found in non-gastrectomized iron deficient subjects (Table 1).

It may thus be concluded that after the Billroth II gastric resection the absorption from an iron salt was reduced in all subjects and more reduced in the subjects with the lowest hemoglobin values. The absorption from ferrous sulphate was more reduced than the absorption of hemoglobin iron which was quite evident only in the subjects with the lowest hemoglobin values.

Thus there is a fundamental difference between non-gastrectomized iron deficient patients who absorb more iron the more severe the iron deficiency and patients with a Billroth II gastric resection who show an inverse relationship between absorption and degree of iron deficiency. The iron deficiency may after a Billroth II resection thus reflect the degree of the absorption defect for iron induced by the operation. However additional factors influence the rate of development of an iron deficiency after a gastric resection e.g. the amount of

stored iron before the operation, the amounts of blood lost and transfused at the operation, other iron losses (menstruations etc.) and the intake of iron from diet or medication. All such factors as well as the time interval to the operation have to be considered in comparative studies of the effect of various resection methods on the iron balance. In the selection of the present material attempts were made to consider most of these factors.

The Billroth I gastric resection was followed by a less severe impairment of the iron absorption than the Billroth II resection. In subjects 1-4 with normal hemoglobin values in the Billroth I group the absorption of iron from ferrous sulphate and from hemoglobin was of the same magnitude as in the normal non-anemic subjects and the absorption ratio ($\text{FeHb}/\text{FeSO}_4$) was about 1 as in the normal subjects. In subjects 5 and 6 who had subnormal hemoglobin values the iron absorption was increased but not to the same extent as in the non-anemic iron deficient blood donors. This indicated that there was a minor defect of the absorption of iron in the anemic Billroth I patients. However, contrary to the Billroth II material the absorption ratio ($\text{FeHb}/\text{FeSO}_4$) was about the same in the Billroth I patients with subnormal hemoglobin values as in the non-gastrectomized iron deficient patients. The very low absorption in subject 7 was probably related to the mentioned dumping symptoms with rapid gastric emptying and rapid transfer of the intestinal contents.

The present study thus indicates that the Billroth I gastric resection may give a minor quantitative absorption defect affecting the iron absorption from both hemoglobin and iron salts. Such a quantitative, but more pronounced defect was observed also after the Billroth II resection. This may be explained by for instance a shorter time of digestion in the stomach, a lower production of hydrochloric acid, and a more rapid transport of the intestinal contents.

The observation of a greater impairment of the iron absorption after the Billroth II than after the Billroth I gastric resection is consistent with the clinical observation that there is a higher frequency of iron deficiency anemia after the Billroth II resection. The main discrepancy compared to previous iron absorption studies is the reduced absorption from ferrous sulphate observed after the Billroth II resection (2-9). The most probable explanation of this discrepancy is the previously mentioned difficulty to establish that the absorption is changed by gastrectomy in the single subject.

The two types of gastric resection affected the iron absorption differently. After both types a reduction of the iron absorption from hemoglobin and from ferrous sulphate was noted (a quantitative defect). After the Billroth II resection a qualitative defect was also observed, i.e. the marked reduction of the absorption from ferrous sulphate. The pathophysiological basis of this difference between the Billroth I and Billroth II gastric resection is probably the exclusion of duo-

denum and a part of the jejunum with the Billroth II resection. Such an exclusion may impair the absorption from an iron salt more than of hemoglobin iron as iron salts are absorbed in the upper part of the intestine in ionized form and thus dependent on pH, while hemoglobin iron is absorbed as an iron porphyrin complex which very likely is unaffected by pH (6-8).

Summary

The iron absorption from ferrous sulphate and hemoglobin was compared in the same subject using a double radioiron method. The study was made in male subjects with a Billroth I or with a Billroth II gastric resection and in a material of non-gastrectomized normal or iron deficient subjects.

A quantitative absorption defect involving both hemoglobin iron and ionized iron was observed in patients with both types of gastric resection. The defect was most pronounced after the Billroth II resection. After this operation there was also a qualitative absorption defect which affected the absorption from iron salts.

The absorption defects seemed to be more pronounced the more severe the iron deficiency, indicating a causal connection.

The observed differences in degree and type of impairment of the iron absorption probably explain the different frequency of iron deficient anemia after the two types of gastric resection.

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The absorption defects seemed to be more pronounced the more severe the iron deficiency indicating a causal connection.

The observed differences in degree and type of impairment of the iron absorption probably explain the different frequency of iron deficient anemia after the two types of gastric resection.

addition to a number of other factors. The enzyme of catalase containing iron in the molecule as prosthetic group is counted among these enzymes.

In view of the observation that desferrioxamine B in addition to eliminating superfluous iron from the organism also produces a stimulating effect upon oxidative metabolic processes (12) and in consideration of the role of iron as catalyser in the electron transfer in these processes the effect of desferrioxamine B upon the enzymatic peroxidative activity of catalase in comparison with the level of serum iron was investigated *in vivo* and *in vitro* in this study. Parallel investigations in the effect of the compound upon the enzymatic oxidative activity of PPD oxidase containing copper in the prosthetic group and ranged with catalase in the group of oxidoreductase were carried out at the same time.

Material and Methods

In experimental series the first series of tests were carried out to establish the effect of intravenous administration of desferrioxamine B on 9 subjects (3 with reduced iron values and 6 with increased serum iron values). Blood samples were collected before and 30 minutes, 1, 3, 5 and 24 hours after the application. The values of iron bound to transferrin, the unsaturated iron binding capacity (IBC) and serum copper were determined. In the second series were the plasma catalase and catalase activity. Two patients suffering from haemochromatosis and two healthy adults as controls were subjected to test in a similar manner. Blood samples in the second series were taken at intervals

equal to those in the first series in addition to transferrin bound iron, IBC and serum copper and enzymatic catalase and PPD oxidase activities we also determined the quantity of total serum iron (iron bound to transferrin together with iron bound to desferrioxamine B).

Investigations *in vivo* were preceded by investigations *in vitro* performed in such manner that trivalent iron desferrioxamine B compound and ferrioxamine compound were added to native serum. Analysis of the enzymatic activity of catalase and PPD oxidase was performed in the native serum as well as in the eluate groups of loaded sera.

Determination of transferrin bound iron was performed according to the method of Hellmeyer and Holzer (2). Copper analyses were made with the modification according to Hoyer et al. (6). The method of Dill (3) was applied in measuring the enzymatic activity of plasma catalase and the Keler-Bafoka (10) modification was employed in determining the catalytic activity of serum PPD oxidase. The determination of total serum iron (transferrin bound iron together with iron bound to desferrioxamine) was carried out according to Keler (8) with the modification that the serum in acid medium was placed for the duration of 15 minutes into a waterbath at 100°C. The determination of unsaturated iron binding capacity (IBC) was performed with modification by Ventura (11).

Experiments and Results

Investigations *in vitro*

In order to exclude the possibility of the compounds desferrioxamine B and ferrioxamine and trivalent iron affecting the enzymatic activity of catalase and PPD oxidase the experiments *in vitro* were performed so that the native serum was loaded with each of the compounds in such manner that the final concentration amounted to 500, 100, 200 and 100 mg in 100 ml of

The Effect of Desferrioxamine B upon the Enzymatic Catalase and PPD-Oxydase Activity in Plasma and Serum

By A HAHN and MIRA KALER BACOKA

Administration of the compound desferrioxamine B for the scope of specific and selective elimination of iron from overstured organs and tissues (1) has lately found wide application in the treatment of various pathologic conditions requiring removal of superfluous iron from the organism. Data have been presented on treatment with the compound administered in cirrhosis of the liver, essential pulmonary siderosis, sideroachrestic anemia, transfusion haemosiderosis and in iron poisoning papers describing the administration of the compound in the treatment of haemochromatosis (2-4, 7, 9) are particularly numerous.

In addition to excretion and elimination of large quantities of iron — up to 90 mg in 24 hours — by way of the urine, clinical improvement in some patients was observed to set in much earlier than could be explained in direct relation with the quantity of iron eliminated (12). The very quick depigmentation of the skin was particularly notable; it would take place as early as two to 3 weeks after treatment

was started although the tissues are then still oversaturated with iron in haemochromatosis. The examination of excised skin has shown that the treatment reduced the melanin content in the basic layer of the skin. Wohler (12) has tried to interpret the phenomenon as probably being the catalytic effect of desferrioxamine B in the sense of inhibition of adrenaline oxidation through adrenochrome to melanin in presence of trivalent iron which mobilized by means of the compound from the tissue during treatment occurs in increased quantities in the circulating plasma. Wohler also believes that the rapid subjective improvement of the patient's condition in the initial stage of treatment could be related not only to the elimination of the superfluous iron but also to the acceleration of metabolic oxidative processes. Iron performs a catalytic function in a function in a series of reactions of redox type in the sense of electronic transfer enzymes which catalyse redox processes are known to play an essential part in these processes in

addition to a number of other factors. The enzyme of catalase containing iron in the molecule as prosthetic group is counted among these enzymes.

In view of the observation that desferrioxamine B in addition to eliminating superfluous iron from the organism also produces a stimulating effect upon oxidative metabolic processes (12) and in consideration of the role of iron as catalyser in the electron transfer in these processes the effect of desferrioxamine B upon the enzymatic peroxidative activity of catalase in comparison with the level of serum iron was investigated *in vivo* and *in vitro* in this study. Parallel investigations in the effect of the compound upon the enzymatic oxidative activity of PPD oxidase containing copper in the prosthetic group and ringed with catalase in the group of oxidoreductase were carried out at the same time.

Material and Method

Investigations *in vivo* in the first series of tests were carried out by means of intravenous administration of desferrioxamine B¹ in 3 subjects, i.e. 3 with reduced, 3 with normal and 3 with increased serum iron values. Blood samples were collected before and 30 minutes, 1, 3, 6 and 4 hours after the application. The values of iron bound to transferrin, the unsaturated iron binding capacity (UIBC) and serum copper were determined in these samples as were the plasma catalase and serum PPD oxidase activities. Two patients suffering from haemochromatosis and two healthy adults as controls were subjected to tests in an analogous manner. Blood samples in this series of tests were taken at intervals

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¹ Desferrioxamine B (Desferal) (Sandoz AG, Basel, Switzerland).

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addition to a number of other factors. The enzyme of catalase containing iron in the molecule as prosthetic group is counted among these enzymes.

In view of the observation that desferrioxamine B in addition to eliminating superfluous iron from the organism also produces a stimulating effect upon oxidative metabolic processes (12) and in consideration of the role of iron as catalyser in the electron transfer in these processes the effect of desferrioxamine B upon the enzymatic peroxidative activity of catalase in comparison with the level of serum iron was investigated *in vivo* and *in vitro* in this study. Parallel investigations in the effect of the compound upon the enzymatic oxidative activity of PPD oxidase containing copper in the prosthetic group and mixed with catalase in the group of oxidoreductase were carried out at the same time.

Material and Method

Investigations in man in the first series of tests were carried out by means of intravenous infusion of desferrioxamine B in 9 subjects of 30-40 years of age with normal and 3 with increased serum iron values. Blood samples were collected before and 30 minutes, 3, 6 and 24 hours after the injection. The values of iron bound to transferrin, unsaturated transferrin capacity (TIBC) and serum copper were determined in the samples as were the plasma catalase and serum $\gamma\gamma$ -glutamate dehydrogenase activities. Two patients suffering from latent iron deficiency and two healthy adults as controls were subjected to the same analogous and control blood samples in this series of tests were taken at intervals

equal to those in the first series. In addition to transferrin bound iron (TIBC) and serum copper and enzymatic catalase and $\gamma\gamma$ -glutamate dehydrogenase activities we also determined the quantity of total serum iron bound to transferrin together with iron bound to desferrioxamine B.

Investigations *in vivo* were preceded by investigations *in vitro* performed in such a manner that trivalent iron desferrioxamine B compound and ferrioxamine compound were added to native serum. Analysis of the enzymatic activity of catalase and $\gamma\gamma$ -glutamate dehydrogenase in the native serum as well as in the free groups of loaded sera.

Determination of transferrin bound iron was performed according to the method of Hellner and Hellner (4). Copper analyses were made with the modification according to Hoyer et al. (5). The method of Hellner (3) was applied in measuring the enzymatic activity of plasma catalase and the Heller-Bach (10) modification was employed in determining the oxidative activity of serum $\gamma\gamma$ -glutamate dehydrogenase. The determination of total serum iron (transferrin bound iron together with iron bound to desferrioxamine B) was carried out according to Heberle (8) with the modification that the serum sample in the cuvette was placed for the duration of 15 minutes into a water bath at 100°C. The determination of unsaturated iron binding capacity (TIBC) was performed with the modification by Ventura (11).

Experiments and Results

Investigations *in vitro*

In order to exclude the possibility of the compounds desferrioxamine B and ferrioxamine and trivalent iron affecting the enzymatic activity of catalase and PPD oxidase the experiments *in vitro* were performed so that the native serum was loaded with each of the compounds in such a manner that the final concentration amounted to 200, 400, 600, 800 and 100 mg in 100 ml of

The Effect of Desferrioxamine B upon the Enzymatic Catalase and PPD-Oxydase Activity in Plasma and Serum

By A HAHN and MIRA KALER BACOKA

Administration of the compound desferrioxamine B for the scope of specific and selective elimination of iron from oversaturated organs and tissues (1) has lately found wide application in the treatment of various pathologic conditions requiring removal of superfluous iron from the organism. Data have been presented on treatment with the compound administered in cirrhosis of the liver, essential pulmonary siderosis, siderochrestic anemia, transfusion haemosiderosis and in iron poisoning, papers describing the administration of the compound in the treatment of haemochromatosis (2, 4, 7, 9) are particularly numerous.

In addition to excretion and elimination of large quantities of iron — up to 90 mg in 24 hours — by way of the urine, clinical improvement in some patients was observed to set in much earlier than could be explained in direct relation with the quantity of iron eliminated (12). The very quick depigmentation of the skin was particularly notable; it would take place as early as two to 3 weeks after treatment

was started, although the tissues are then still oversaturated with iron in haemochromatosis. The examination of excised skin has shown that the treatment reduced the melanin content in the basic layer of the skin. Wöhler (12) has tried to interpret the phenomenon as probably being the catalytic effect of desferrioxamine B in the sense of inhibition of adrenaline oxidation through adrenochrome to melanin in presence of trivalent iron which, mobilized by means of the compound from the tissue during treatment, occurs in increased quantities in the circulating plasma. Wohler also believes that the rapid subjective improvement of the patient's condition in the initial stage of treatment could be related not only to the elimination of the superfluous iron but also to the acceleration of metabolic oxidative processes. Iron performs a catalytic function in a function in a series of reactions of redox type in the sense of electronic transfer enzymes which catalyse redox processes are known to play an essential part in these processes, in

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Material and Method

Investigations *in vivo* in the first series of tests were carried out by means of intravenous administration of desferrioxamine B¹ in 9 subjects i.e. 3 with reduced, 3 with normal and 3 with increased serum iron values. Blood samples were collected before and 5 minutes, 1, 3, 7 and 24 hours after the application. The values of iron bound to transferrin, the unsaturated iron binding capacity (UIBC) and serum copper were determined in these samples as were the plasma catalase and serum PPD oxidase activities. Two patients suffering from haemochromatosis and two healthy adults as controls were subjected to tests in an analogous manner. Blood samples in this series of tests were taken at intervals

equal to those in the first series in addition to transferrin bound iron (TIBC) and serum copper and enzymatic catalase and PPD oxidase activities we also determined the quantity of total serum iron (iron bound to transferrin together with iron bound to desferrioxamine B).

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¹ Desferrioxamine B (Desferal) (Ciba AG, Basel, Switzerland).

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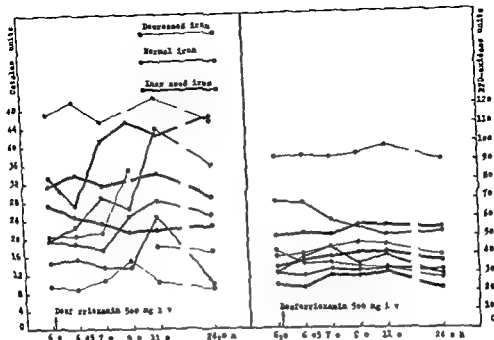


Table 2 Values of enzymatic activity of catalase and PPD oxidase in plasma and serum prior to and after application of Desferrioxamine B in subjects with different initial serum iron values

The results of the determination of PPD oxidase activity — as seen in table 2 — showed no essential change after application

Two patients with haemochromatosis (table 4) and two normal subjects as controls (table 3) were treated in analogous manner in the second series of experiments. Determination of total serum iron i.e. transferrin bound iron together with iron bound to desferrioxamine B was additionally performed in this series of experiments. The results of the control tests (table 3) and in part also the results obtained in two patients with haemochromatosis (table 4) show analogy with the results of the first series of experi-

ments i.e. a marked reduction of transferrin bound iron 5 minutes after application and practically unchanged values of serum copper and PPD oxidase after application. The values of total iron after administration of desferrioxamine B underwent no essential change in the control tests but showed marked increase in the patients with haemochromatosis. A notable increase in catalase activity after the application regardless of the concentration of total iron and of transferrin bound iron was registered in both the patients and the controls. Equal to the first series of tests the control tests in the second series showed an obvious rise in UIBC values immediately on the ad-

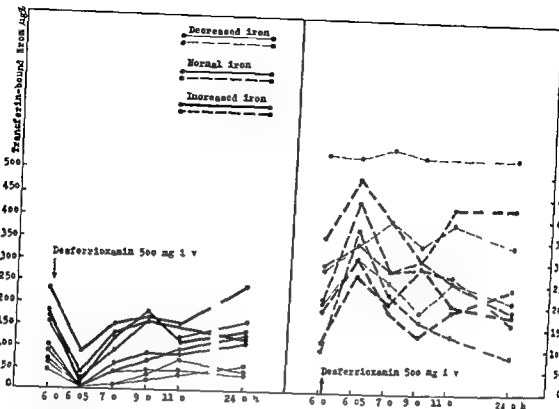


Table 1 Values of transferrin bound iron and unsaturated iron binding capacity (UIBC) in the serum prior to and after i.v. application of the compound Desferrioxamine B 500 mg in subjects with different initial values of serum iron

serum Examination of the enzymatic activities of catalase and PPD oxidase showed no essential difference between the native serum and the loaded one

Investigations in vivo

The results of the first series of experiments, presented in table 1, showed that a marked reduction of transferrin bound iron had taken place in all examined subjects 5 minutes after application of the compound. Furthermore in the subjects with reduced initial serum iron values the serum iron became completely bound to desferrioxamine B so that the determinations in these subjects showed nil values 5

minutes after administration. Simultaneously there was an obvious increase in all examined subjects of the unsaturated iron binding capacity (UIBC) reflecting the condition of serum transferrin free from iron. The level of serum copper underwent no essential change in the course of the experiment.

The values of enzymatic catalase activity presented in table 2 indicate that enzymatic activity increased in 6 of the investigated subjects 1, 3 or 5 hours after the application, regardless of the serum iron level but these values returned to the range of initial results after 24 hours. In three instances catalase activity remained practically unchanged throughout the experiment.

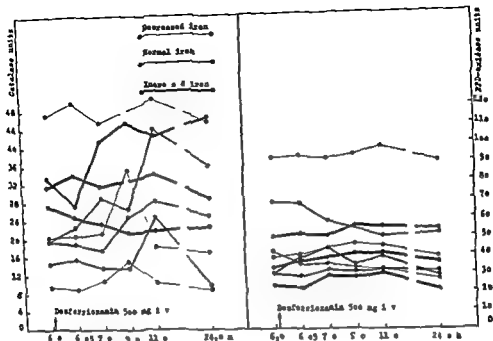


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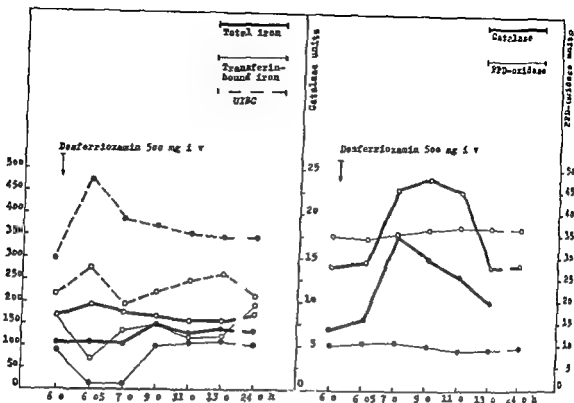


Table 3 Values of total serum iron transferrin bound iron and UIBC and enzymatic activity of catalase and PPD oxidase prior to and after i.v. application of Desferrioxamine B 500 mg in two normal subjects

ministration of the compound in one of the patients with hemochromatosis the reduced UIBC values increased, the other patient showed no capacity for binding serum iron throughout the entire experiment. The latter patient was again subjected to the investigation after having been treated with desferrioxamine for several months. The results of the second investigation resemble as to values for transferrin bound iron total iron PPD oxidase and catalase activity to the results obtained in the first experiment made with this patient. The only difference was observed in UIBC values 15 min after administration the reason being that the partial depletion of iron from

the tissues caused by the long lasting treatment was registered in the second test with increased values of UIBC due to transferrin freed from iron. As soon as 1 hour after the administration of desferrioxamine B in this test UIBC values had returned to normal.

Discussion

The results of this study show that desferrioxamine B administered intravenously in healthy persons is well tolerated in subjects with reduced or with increased iron levels and patients with hemochromatosis binds immediately on application i.e. in 5 min the circulating transferrin bound plasma iron

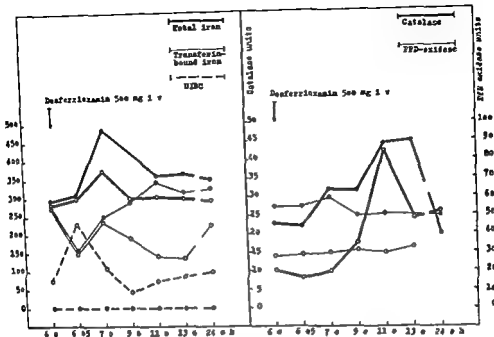


Table 4 Values of total serum iron transferrin bound iron unsaturated iron binding capacity and enzymatic activity of catalase and PPD oxidase in two patients with haemochromatosis prior to and after application

thus causing marked decrease of this iron. Furthermore, in the examined subjects whose initial iron levels before the administration of desferrioxamine B had been below normal values, the determination of transferrin bound iron values 3 min after administration was nil. This signifies that the desferrioxamine B compound *in vivo* in these cases directly binds all available plasma iron before the compound can diffuse in the tissues. The results of UIBC determination reflect by raised values the condition of plasma transferrin freed from iron immediately on administration of desferrioxamine. The amount of 500 mg of desferrioxa-

mine B is known to be capable of binding about 45 mg of iron — it is hence understandable that a reduction — and in case of anemia even complete disappearance — of transferrin bound iron which becomes bound to desferrioxamine B is to be expected to take place in total plasma.

In his earlier investigations Wohler observed that the total iron level in patients with hemochromatosis rose from 1 hour after application onwards. (2) the same effect was registered in this study. No increase of total iron after administration was noted in healthy subjects. The values obtained in the course of the investigation in

determinations of copper and of PPD-oxidase activity show that the application produces no change

Conversely, in the majority of examined subjects, in two patients with haemochromatosis and in two healthy persons the values for enzymic catalase activity show an increase 1, 3 and/or 5 hours after administration. The increase in catalase activity is not connected with the amount of circulating plasma iron since it was found in the normal subjects as well as in the other ones in whom no increase in total iron was registered and in those who had haemochromatosis when significant increase of total iron was registered. These data are complemented by the results of *in vitro* investigations in the activity of enzymic catalase and PPD oxidase in serum loaded *in vitro* with the compound desferrioxamine B, ferrioxamine and with iron respectively. Enzymic activity was found not to be changing in loaded serum *in vitro* when compared with the activity in not loaded serum.

Although iron constitutes the prosthetic group of the peroxidative catalase enzyme these results on basis of experiments performed *in vivo* and *in vitro* allow for the conclusion that the increase of enzymic activity of catalase *in vivo* after administration of the compound desferrioxamine B could not be brought into causative relation with the quantity of circulating plasma iron regardless of whether the iron was bound to transferrin or to desferrioxamine B. The increased enzymic catalase activity observed in this study on administration of desferrioxamine B

probably may be connected with the other accelerated oxidative metabolic processes described also by other authors in the course of treatment with the compound desferrioxamine B (12)

Conclusion

The concentration of transferrin bound iron, the iron binding capacity (UIBC), copper, as well as the enzymic activity of catalase and PPD oxidase were studied prior to and after application of the compound desferrioxamine B (500 mg) in two patients suffering from haemochromatosis, in two normal controls and in 9 subjects with reduced, normal and increased serum iron levels respectively.

The results showed that as early as 5 min after intravenous administration of the compound there ensued marked reduction of transferrin bound iron with a concurrent sudden increase in UIBC. One hour after administration the values for transferrin bound iron and UIBC approximated those registered before loading to return to initial values after 24 hours. The application of the compound produced no effect upon the level of copper and the enzymic activity of PPD oxidase in all examined subjects.

It was observed in two patients with haemochromatosis that the level of total serum iron (transferrin bound iron with iron bound to desferrioxamine B) showed a sudden rise after the administration while the values for total iron ranged in physiological variations in the control test.

Enzymic catalase activity in 8 of

11 studied subjects and in both patients with haemochromatosis regardless of the quantity of iron in circulating plasma 1, 3 and/or 11 hours after administration of desferrioxamine 11 showed increased activity and consecutive reduction to initial values after 24 hours

The in vitro loading of the serum with the compound desferrioxamine B ferrioxamine and trivalent iron respectively indicates that the loading in vitro does not change the enzymatic activity of PPD oxidase and catalase

We are indebted to pharmacist Andrina Stojanoviš and laboratory technician Josipa Franjlović for technical collaboration

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Simplified method for determining carbon monoxide hemoglobin saturation in diagnosis of hemolytic disorders

By KARL GYDELL

The value of carbon monoxide hemoglobin (COHb) determinations in the diagnosis of hemolytic states is well documented (12, 7, 4, 5, 2). An advantage of this test compared with the isotope methods (Cr^{51} and Fe^{59}) is that it can be used repeatedly *ie* for evaluating the effect of a given therapy. The COHb method has been used mainly in research work concerning hemolytic problems. It is not used so widely in clinical routine work. There are probably several reasons for this restricted use of the test. One of the most important is probably of technical nature *viz* the complicated methods for determining the very low concentrations of endogenous carbon monoxide. The method that has been used longest is the 'thermochemical' or 'Hopcalite method' originally described by Sjöstrand (11) and later modified by Lindnerholm and Sjöstrand (8). The thermochemical method is however rather complicated and laborious. For optimal function the system should

preferably operate continuously. The author used the method for two years and found it very accurate. The methodological error was found to be about $\pm 0.03\%$ COHb (6). There are also colorimetric methods available for determining carbon monoxide in low concentrations. A very sensitive method is described by Shepherd (10). In that test a CO sensitive substance is enclosed in special glass tubes which are commercially available¹. Andersson and Dahlström (1) have described a method in which the colour change obtained when the tubes are exposed to a CO containing gas sample can be read photometrically. The author has used this method with slight modifications in a large number of investigations and has found it reliable and handy (6). A disadvantage of this method is that every new set of indicator tubes must be calibrated which is a rather laborious and time consuming procedure.

¹ In Sweden from F&B Produkter Fabrikssaklabolag Stockholm



Fig 1 The Drager suction pump connected to an indicator tube

A simple and rather accurate variant of the colorimetric method for determining low CO concentrations is described below.

Alveolar air samples from the subjects to be investigated were obtained by rebreathing pure oxygen in a rebreathing apparatus designed by AGA Lidinö (AGA MF 1500).

The CO concentrations in the rebreathed gas samples were measured with special indicator tubes (Type a/b) manufactured by Dragerwerk Lubeck (3). These test tubes covers a measuring range from 0 to 200 ppm. The gas sample is sucked through the tube with a pump (Fig. 1) and a colour change is obtained in the indicator substance which contains a mixture of iodine pentoxide and sulphuric acid. The gas volume needed for one analysis is one liter which can be sucked through the indicator tube in two minutes. The final level of the colored

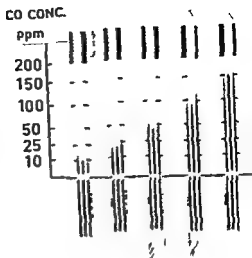


Fig 2 Propagation of the colour zone in a Drager test tubes exposed to carbon monoxide in concentration steps from 100 to 10 ppm

zone is read against the scale of the tubes.

Results and discussion

Fig. 2 gives a reproduction of a test tubes exposed to CO mixtures containing from 100 to 10 ppm and illustrates the levels of the coloured zones. The results of two double determinations are given in Fig. 3. In order to compare the readings of the Drager CO detector tubes with the colorimetric method according to Andersson and Dahlström (1) estimations were made simultaneously in 31 tests at various CO concentrations. The results are given in Fig. 4. The vertical lines in the diagram mark the range within which the various examiners considered the colour to cease. The stan-

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¹ In Sweden from LKB Produkter Fabrikationsbolag Stockholm

the blood can be calculated (3) By a slight modification of an ordinary spirometer for BMR investigations it may be possible to perform the re-breathing procedure without any special apparatus

Summary

A simplified method for measuring carbon monoxide concentrations in gas samples after rebreathing pure oxygen with the use of Dräger CO detector tubes (Type a/b) with a measuring range of 5 to 200 ppm is described. The corresponding carbon monoxide hemoglobin concentration in the blood is calculated with Haldane's equation. A comparison with another method for carbon monoxide determination indicates that the method described is accurate enough for detecting significant hyperhemolysis in clinical routine work.

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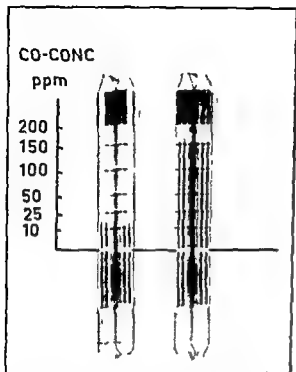


Fig 3 Two pairs of double determinations at different carbon monoxide concentration. In the left pair there is a slight discrepancy between the positions of the colour levels. No obvious difference can be seen in the right pair.

standard deviation of these determinations was ± 27 ppm. Complete calculation of the COHb level in the blood from the CO concentration in alveolar air according to Haldane's equation requires estimation of the oxygen concentration in the gas samples (11, 13). Provided that the rebreathing procedure is performed in a correct manner so that no leakage occurs in the connection between the patient and the aggregate, the oxygen concentration after a rebreathing time of 15 minutes in pure oxygen varies but little from 95 volume per cent. Introduction of this value as a constant in Haldane's equation gives a factor of 0.025

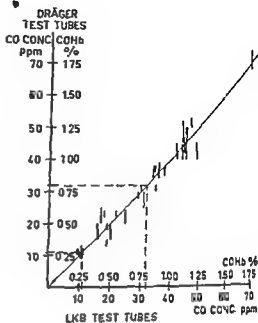


Fig 4 Relations between photometric CO determinations with "LKB" test tubes (abscissa) and direct reading from the same gas sample in Dräger's test tubes (ordinate). Besides the CO concentrations the corresponding COHb values are given. The interrupted lines denote the normal ranges of the endogenous CO and COHb values.

which has been used here for converting a given CO concentration in ppm to COHb in per cent saturation ($\text{COHb per cent} = \text{CO}_{\text{ppm}} \times 0.025$). The aforementioned reading error of ± 27 ppm thus corresponds to 0.07 per cent COHb. This error is much larger than that of the two other methods mentioned above, but the accuracy of the Dräger CO detector tubes seems to be sufficient for discovering hyperhemolysis in routine clinical work. These tubes can also be used for direct analysis of the CO concentration in expired air in suspected CO intoxication (9). From the CO concentration obtained a corresponding COHb level in

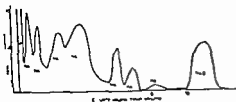


Fig 1 Radioactivity curve from a partial hydrolysate of red cell ghosts separated on a Dowex 50 (8% DVB) column. Incubation time 16 hrs in 18 C



Fig 2 Radioactivity curve (the continuous line) of the material from peak No 5 Fig 1 separated on a Dowex 1 (2% DVB) column. The broken line represents formic acid concentration

Only UV absorbing material was found in subfraction 6 (fig 2). The material from peak III ("phosphorylcholine") could also be separated into 6 fractions by Dowex 1 column chromatography. Phosphopeptides were found in subfraction 1 and 4. Number 2 and 3 contained phosphorylcholine and 5 and 6 both contained UV absorbing material (fig 3).

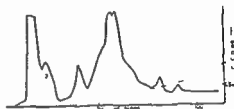


Fig 3 Radioactivity curve of the material from peak No 8 Fig 1. See legend for Fig 2

The UV absorbing material was further purified by high voltage electrophoresis using Whatman No 3 paper. The electropherograms were run in the Wicand Pfeiderer apparatus at 260 V and 35 mA for 60 min at 4-6 C using a 0.05 M citric acid sodium citrate buffer of pH 3.6 (13). The UV absorbing spots were then eluted from the electropherograms with water. Samples from the eluate were analyzed for ribose (10) and phosphorus (11). The remaining material in amounts not exceeding a UV absorbance value of 1.0 cm at 260 mμ was all hydrolyzed in 0.5 ml of concentrated formic acid for 10 min in sealed tubes in 170 C. The hydrolysates were analyzed by thin layer chromatography together with the reference

substances adenine, guanine, uracil, methylcytosine and cytosine as described by Josefsson (12) with slight modifications. In some experiments the eluted material was concentrated *in vacuo* and then dissolved in 0.01 N HCl and the UV spectrum was measured in a Perkin Elmer Model 137 UV spectrophotometer. The UV spectrum at pH 2.0 for the substances are illustrated in fig 4 and 5.

In thin layer chromatography the UV absorbing material from the electropherograms in all cases ran parallel with the reference cytosine. In addition the UV spectrum of all substances was in good agreement with that of cytidine (13). Attempts were made to estimate ribose and phosphorus in the eluted UV absorbing spots but the values obtained were

The Occurrence of a Metabolically Active Cytosine compound in a Protein Fraction from Human Erythrocyte Ghosts

By GUNNAR ROXQUIST and GUNNAR ÅGREN

The uptake of labeled inorganic phosphate ($^{32}\text{P}_i$) by human erythrocyte ghosts has previously been studied (1, 2). It was also shown that ^{32}P was incorporated into phosphopeptides as well as into phosphorylethanolamine and phosphorylcholine. This process was rather slow and the labeling of the phosphopeptides preceded that of phosphorylethanolamine and phosphorylcholine.

It has also been reported (3, 4) that whole human erythrocytes incorporate $^{32}\text{P}_i$ mainly into phosphatidic acid. Rowe (5) in studying the metabolism of unfractionated blood cells observed a more uniform ^{32}P labeling of the cellular phospholipids but it has been claimed (6), that these results likely are not to be attributed to the erythrocytes. Thus James et al (7), Marks et al (8) and Buchanan (9) all claim that any synthesis of lipid in blood is a result of white cell and reticulocyte activity.

Some data are now reported as evidence for a biosynthesis of phospholipids in the red cell membrane itself.

Ghosts were prepared from human

erythrocytes which had been separated from most of the white cells (2). They were incubated for various periods with $^{32}\text{P}_i$ and then precipitated with trichloroacetic acid (1, 2). The precipitate was dried with ethanol and the lipids were removed by extraction twice with ethanol/ether at 45°C followed by ether.

The residual "protein" fraction was partially hydrolysed in 2 N HCl for 20 hrs and the hydrolysate was applied to a Dowex 50 column and eluted with 12 column volumes of 0.01 N HCl. The elution pattern is shown in fig 1*. All peaks contained labeled phosphopeptides but peak 5 and 8 contained in addition labeled phosphorylethanolamine and phosphorylcholine respectively.

All these peaks could be further separated into several subfractions on a Dowex 1 formate column with gradient elution $0 \rightarrow 1\text{ M}$ formic acid. Thus peak 5 ("phosphorylethanolamine") was further separated into 6 subfractions of which 1 and 2 contained phosphopeptides. Phosphorylethanolamine was found in 3 and 4

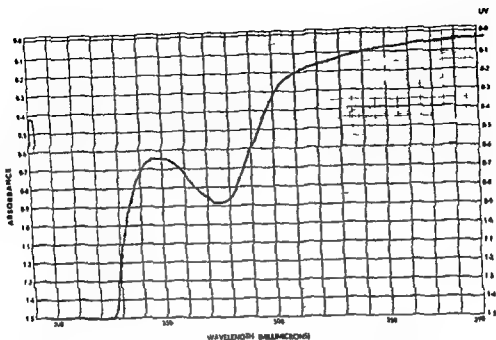


Fig 5 UV spectrum at pH 2.0 of the eluted UV absorbing spot in the electropherogram of fraction 6 Fig 2

ATP necessary for a formation of phosphorylethanolamine and phosphorylcholine is also formed in the red cell membrane (16, 17)

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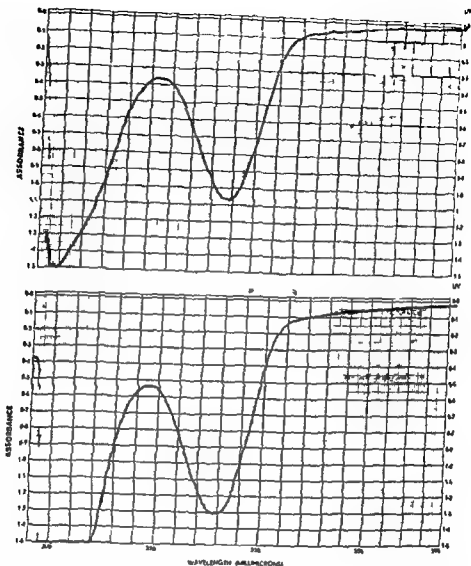


Fig 4 UV spectra at pH 2.0 of the eluted UV absorb₂₆₀ spots in the electro pherograms of fraction 5 (upper) and fraction 6 (lower) Fig 3

rather low and too fluctuating to give reliable information

It seems that the found cytosine compound is rather hardly bound to the stromal proteins and since cytosine was isolated as a compound labeled with ^{32}P , it seems probable that this compound might be cytidylic acid. We have previously reported the isolation of comparatively large amounts of phosphorylethanolamine

and phosphorylcholine from the same source (2). It is therefore suggested that the isolated cytosine compound together with the latter compounds are involved in the synthesis of phosphatidylethanolamine, lecithin and sphingomyelin in the red cell membrane in the same pathway as described by Kennedy and Weiss (14, 15) for other tissues.

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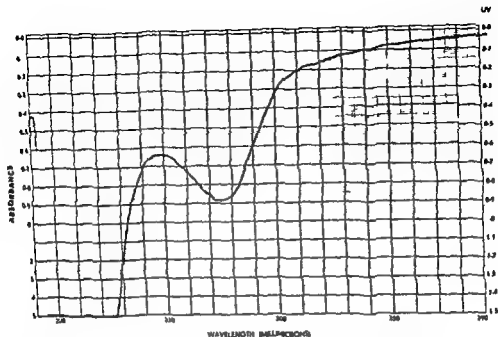


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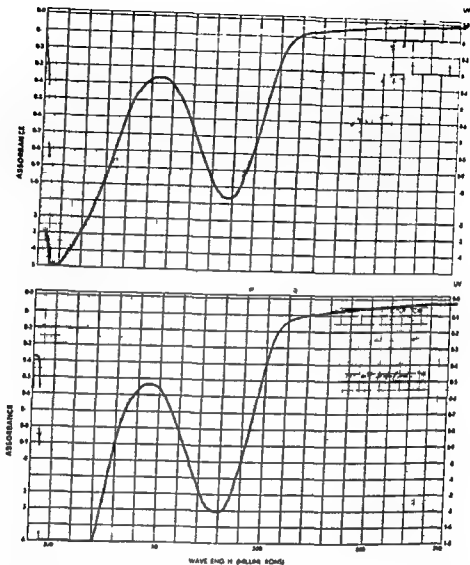


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It has recently been shown that the

établirent la notion d'agranulocytose périphérique insistèrent sur l'aspect normal du myélogramme. Normale et même hyperplasique initialement au point que cette hyperplasie granulocytaire était comparée à l'érythroblastose médullaire des anémies hémolytiques. Les images d'hypoplasie granulocytaire étaient tenues pour rares et toujours tardives (17). Les études récentes (1) montrent qu'au moins dans la grande majorité des cas l'évolution des myélogrammes est toute différente et même exactement contraire. 1) l'atteinte du tissu granulopoïétique médullaire est extrêmement fréquente sinon constante au stade initial. 2) ces lésions médullaires sont variées mais le désordre le plus commun est le blocage l'arrêt de maturation au stade promyélocyttaire. 3) ces lésions sont éphémères et très rapidement corrigées (c'est alors mais alors seulement que l'on note l'hyperplasie granulocytaire).

Ainsi ces images médullaires des agranulocytoses du pyramidon sont issues originales et doivent être distinguées aussi bien des images normales décrites peut être par erreur en 1933 que des lésions médullaires des aplasies dues aux antimétaboliques qui ne sont jamais ou presque jamais aussi vite réparées.

Le chronologique véritable ne permet pas de relier les explications des lésions médullaires qu'on donne ni même ni l'atteinte tardive des granulocytes médullaires par la substance noire ni l'épuisement médullaire se condure. Elle s'accorderait bien avec l'hypothèse d'une altération précoce

du tissu granulopoïétique médullaire. Il est difficile, en l'état actuel de discerner si les lésions granulocytaires initiales sont purement médullaires ou si ces lésions sont à la fois médullaires et périphériques ou encore si une lésion initialement périphérique est suivie très rapidement par un mécanisme qu'il faudrait préciser d'un arrêt de la maturation médullaire. On doit en tout cas tenir compte de la précocité et de l'allure de ces lésions médullaires dans la discussion du mécanisme de l'agranulocytose du pyramidon. On doit en tenir compte aussi dans la discussion du diagnostic. Ces moelles promyélocytaires sont parfois fort étonnantes. Le diagnostic de leucémie atypique à promyélocytes peut être évoqué. Les promyélocytes de l'agranulocytose du pyramidon sont en règle générale normaux et très différents des promyélocytes leucémiques altérés avec leurs grosses granulations. Les difficultés n'en sont pas moins parfois très grandes. Les meilleurs cytolologistes ont pu se tromper et c'est avec bonheur que l'on voit la maturation granulocytaire survenant les myélogrammes ultérieurs démentir le cruel diagnostic initial.

L'importance de la plasmocytose médullaire peut être responsable d'autres difficultés de diagnostic. La plasmocytose médullaire fréquente mais habituellement modérée et isolée peut parfois être forte être accompagnée par une plasmocytose sanguine par de franches modifications des protéines sériques. À côté de la plasmocytose une prolifération de cellules réticulaires plus ou moins orien-

Remarques sur l'Agranulocytose du Pyramidon

Par JEAN BERNARD

Une part importante de l'oeuvre du Professeur Jan Wildenstrom est consacrée à l'étude de l'action des médicaments sur le sang. C'est à ce propos que nous apportons ici en hommage à Jan Wildenstrom ces quelques remarques sur l'agranulocytose du pyramidon. Ces remarques sont fondées sur l'examen des cas suivis dans notre Institut à Paris de 1955 à 1965.

Vers 1952 la description de l'agranulocytose du pyramidon paraissait définitive comme semblait simple et assurée l'explication qu'on proposait de son mécanisme. Les expériences d'Ackroyd ou plutôt l'interprétation qui en découlait avaient été transposées des plaquettes et du sedormid aux leucocytes et au pyramidon (bien que l'agglutination des leucocytes n'était observée qu'avec le serum prélevé en pleine crise et que dans aucun cas on n'ait pu jusqu'à un cas récent (15) restaurer le pouvoir agglutinant d'un serum prélevé ultérieurement en lui ajoutant du pyramidon (18) alors qu'il est régulièrement le cas par exemple dans les purpuras du sedormid). L'agranulocytose du pyramidon pense-t-on

alors est une agranulocytose périphérique liée à l'atteinte des éléments granuleux dans le sang circulant, elle se sépare par là des agranulocytoses centrales médullaires, provoquées par les médicaments myélotoxiques comme les moutardes, le benzène le chloramphenicol. Elle est pure purement leucocytaire les globules rouges et les plaquettes restent normaux alors qu'ils sont presque toujours altérés dans l'agranulocytose centrale simple symptôme d'une aplasie médullaire globale. Elle est expliquée par l'hypothèse immuno-allergique alors que les aplasies médullaires médicamenteuses celles par exemple du chloramphenicol demeurent souvent obscures. Elle peut être guérie par un traitement vigoureux et urgent. Elle devrait ainsi être prévenue grâce à la connaissance de sa cause et de sa physiopathologie. Cette agranulocytose du pyramidon qu'on se représente ainsi en 1952 comme une agranulocytose périphérique pure simple curable risquerait d'être prévenue qu'est-elle devenue en 1965?

Les descriptions de 1952—1955 qui

conditions étiologiques. L'induction des accidents par une toute petite dose s'accordent très bien avec l'hypothèse immuno-allergique. On est d'abord tenté d'accuser l'insuffisance des techniques (mais elles se sont améliorées quoique bien des techniques sérologiques n'aient pas encore été mises en oeuvre) ou le retard de l'examen (mais il est précoce avec la dose test). Mais peut-être les difficultés tiennent-elles au fait que l'on ne étudie ni les substances vraiment responsables ni la cellule vraiment touchée. Ce n'est peut-être pas le pyramidon entier mais plutôt tel ou tel de ses catécholites qui induit la réaction. Les recherches entreprises dans cette direction pourraient être développées. Ce n'est peut-être pas le polynucléaire neutrophile mur mais plutôt l'un de ses précurseurs qui est atteint le premier. Les données cliniques et cytologiques la lésion précoce de la moelle érythropoïétique, l'altération légère des autres séries pourraient s'accorder avec cette explication. Les méthodes immunologiques qui ont rendu tant de services dans l'étude des globules du sang ne sont que malaisément applicables aux cellules médullaires. De nouvelles techniques sont à l'étude. Améliorées elles permettront de vérifier ou d'infirmer cette hypothèse de travail.

Le pyramidon peut-il outre l'anémie nucléocytaire provoquer des cytopénies chroniques? Il n'est pas rare dans l'histoire des malades avec cytopénies chroniques de trouver des prises répétées de pyramidon. Il est difficile d'affirmer leur intervention et le craignant de préciser le mode

1) action directe du pyramidon sur les cellules granulocytaires 2) mécanisme allergique la leucopénie étant entretenue par des absorptions fréquentes du médicament contre lequel s'est développé un anticorps, 3) formation d'autoanticorps. Dans une observation d'anémie hémolytique au pyramidon (3) le serum malade est capable d'agglutiner des hématies normales ayant subi un contact prolongé avec le médicament puis trypanisées et tout se passe comme si le pyramidon altérait l'hématie la rendant antigénique pour elle-même. Quelques constatations sérologiques ont permis récemment d'envisager un mécanisme analogue pour certaines leucopénies chroniques (8). Chez certains malades dont le passé pathologique chargé a entraîné un long usage médicamenteux (névropathies rhumatisantes) ou après une intoxication médicamenteuse intense on constate parfois à l'aide des tests directs et indirects de consommation de l'antiglobuline l'apparition de substances gamma globuliniques ayant une spécificité anti-leucocytaire. L'altération médicamenteuse est peut-être capable de provoquer l'apparition parfois transitoire d'auto-anticorps.

La découverte du mécanisme immuno-allergique des cytopénies médicamenteuses a donné bonne conscience et confort intellectuel aux hématologistes. Conscience et confort peut-être prématurés. Car de très importants problèmes ne sont pas résolus. Des dizaines de milliers de personnes en France seulement consomment

tees vers le plasmocytome peut être trouvée, des adénopathies infiltrées de cellules réticulaires et de plasmocytes ont été à titre exceptionnel observées (4, 5). Entre agranulocytose médicamenteuse et sarcomatose, le diagnostic peut être très malaisé. Certes l'étude cytologique attentive permet le plus souvent de poser le diagnostic exact que l'évolution confirme. Jusqu'à présent, l'avenir de ces malades n'est bon. L'hypothèse qui admet des liens entre agranulocytose et myélome n'a pas été confirmée par les faits (11). On comprend à la lecture de certains documents qu'elle ait été proposée.

On crut d'abord que les lignées érythrocytaires et plaquettaires étaient absolument intactes que la lignée granulocytaire était seule touchée. En fait, l'étude des observations met en évidence une atteinte des autres séries rouge et plaquettaire. Cette atteinte est légère, passagère, reconnue seulement par une analyse attentive (1). La durée de vie des divers éléments du sang peut expliquer que cette atteinte soit parfois méconnue. La nature des troubles, en particulier la réticulocytopenie témoignent en faveur d'une altération des tissus érythropoïétiques et thrombopoïétiques médullaires. L'interprétation de ces troubles est malaisée et on ne sait s'il faut leur attribuer une action directe de la substance nocive sur la moelle (et sur quel élément médullaire?) ou envisager d'autres hypothèses.

Les retouches ainsi apportées aux premières descriptions permettent de tracer un tableau qui a une certaine valeur diagnostique. Une agranulocy-

tose profonde, accompagnée d'une très discrète insuffisance rouge et plaquettaire, d'un arrêt passager de la maturation granulocytaire, surtout d'un arrêt promyélocytaire, évoque le empoisonnement par le pyramidon.

Quel est le mécanisme exact de l'agranulocytose du pyramidon? Le leucocyte participe-t-il activement à la constitution de l'antigène formant avec le pyramidon un complexe qui devient antigénique ou ce leucocyte n'est-il atteint que passivement par un conflit immunologique qui se déroule à sa surface? (16, 19)

Depuis dix ans les immunologistes discutent sans s'accorder et, comme au temps de la logique formelle, alignent en face de chaque hypothèse les arguments "pro et contra". Les controverses sont brillantes, elles seraient cependant plus fondées si l'anticorps qui est leur objet était habituellement mis en évidence. Il n'en est rien et dans la grande majorité des observations récentes il n'a pas été possible de démontrer la présence de cet anticorps. Dans un seul cas, il a été possible de démontrer un anticorps actif seulement en présence du pyramidon (15). Cet échec habituel a souvent été noté aussi par les médecins exerçant dans les pays dont la morale permet (ouvertement ou hypocritement) par l'administration de dose test de mettre délibérément en danger la vie de leur prochain, en provoquant une nouvelle agranulocytose. Ces échecs sont d'autant plus surprenants que l'allure clinique de l'agranulocytose du pyramidon, sa violence et sa régression ses-

conditions étiologiques. L'induction des accidents par une toute petite dose s'accordent très bien avec l'hypothèse immuno allergique. On est d'abord tenté d'accuser l'insuffisance des techniques (mais elles se sont améliorées quoique bien des techniques serologiques n'aient pas encore été mises en oeuvre) ou le retard de l'examen (mais il est précoce avec la dose test). Mais peut être les difficultés tiennent elles au fait que l'on n'étudie ni les substances vraiment responsables ni la cellule vraiment touchée. Ce n'est peut être pas le pyramidon entier mais plutôt tel ou tel de ses catabolites qui induit la réaction. Les recherches entreprises dans cette direction pourraient être développées. Ce n'est peut être pas le polynucléaire neutrophile mur mais plutôt l'un de ses précurseurs qui est atteint le premier. Les données cliniques et cytologiques la lésion précoce de la moelle granulopoïétique. L'altération légère des autres séries pourraient s'accorder avec cette explication. Les méthodes immunologiques qui ont rendu tant de services dans l'étude des globules du sang ne sont que malaisément appliquées aux cellules médullaires. D'autres techniques sont à l'étude. Améliorées elles permettront de vérifier ou d'infirmer cette hypothèse de travail.

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ment chaque année du pyrimidon ou l'une des cent spécialités en contenant. Quelques unes seulement seront victimes de la drogue. Les victimes mêmes ne sont pas égales victimes. L'immunisation à un même médicament varie d'un individu à un autre. Ces variations peuvent porter sur le type serologique d'anticorps, sur sa spécificité. Le pyrimidon peut provoquer l'intolérance l'apparition d'un puissant anticorps de type réaginique entraînant des accidents cutanés sans leucopénie (12) l'intolérance l'apparition d'un anticorps leucopénisant agglutinant les leucocytes en présence de pyrimidon (15). La spécificité des anticorps allergiques cytopénisants varie d'un malade à un autre. Tel produit provoquera selon le malade, une anémie hémolytique, un purpura thrombopénique ou une leucopénie. Cette diversité, l'inégale sensibilité des êtres humains au pyrimidon seraient bien expliquées par des facteurs héréditaires, par une prédisposition constitutionnelle. Jusqu'à présent aucun fait positif n'a été rapporté qui témoigne en faveur de cette hypothèse. Mais ces études pharmacogénétiques ont été quinquées. Il serait important de chercher dans les familles des malades des sensibilisations latentes dans le cas de contact familial avec un produit. De telles sensibilisations latentes découvertes par un examen fortuit ont été récemment signalées (14). Il est utile de rappeler certaines constatations expérimentales montrant que la capacité de sensibilisation à un même antigène artificiel est transmise héréditairement (13). Il est difficile de prévoir la nature de ce

désordre héréditaire prédisposant anomalie dans le catabolisme même du médicament, anomalie de la constitution des protéines sur lesquelles le médicament ou sa métabolite pourra se fixer.

Ainsi en l'état actuel, la prévention de l'agranulocytose du pyrimidon ne peut se fonder, comme celle des anémies hémolytiques enzymopriées sur le dépistage biochimique des individus fragiles. Cette prévention repose sur la limitation de l'emploi du pyrimidon. Limitation générale et interdiction de l'initiation aux individus victimes une fois de la drogue. Cette interdiction ne peut être fondée que sur un diagnostic assuré. Les médecins avertis savent toute l'importance d'une enquête minutieuse, patiente, répétée, prolongée qui finit par arracher au malade le souvenir de comprimés de suppositoires oubliés ou négligés. Mais la diversité des formes médicamenteuses et de leurs appellations entrave souvent l'enquête et en compromet le résultat. Comment le médecin le plus prudent peut-il connaître cette liste mouvante qui on lui dit plus haut comprend plus de cent noms pour le seul pyrimidon. Un dictionnaire est nécessaire (2) qui fournit le nom de tous les coupables et vient utilement orienter l'interrogatoire et la prévention. L'effort efficace des organismes internationaux ordonne la nomenclature mais rien ne limite la multiplication des préparations commerciales. Ce désordre entraîne l'usage l'abus presque inconscient d'un médicament parfois dangereux. Une double limitation scientifique et pratique réduisant le vo-

cabulaire et réduisant l'emploi de la drogue est bien souhaitable

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Transient neutrophil agranulocytosis in a newborn with leucocyte antibodies of type anti-8a in the mother

By C F HÖGMAN, G LILIENBERG and B VAHLQUIST

Transient neonatal granulocytopenia was described in 1950 by Slobody et al (26) and in 1951 by Lehdorff (16). This condition is characterised by granulocytopenia in the newborn period usually appearing a few days after birth and generally remitting within the course of one to two months. Infections of various degrees may be found during this period but in many cases there is a complete lack of clinical symptoms. In the first publication by Slobody et al the possibility of an isoimmunisation as the cause of the granulocytopenia was suggested and later positive evidence for such a mechanism was obtained.

In the literature several cases with neonatal granulocytopenia have been described where leucocyte antibodies were indicated as a possible cause (1, 2, 7, 8, 10, 15, 17, 24, 27). The syndrome has been reviewed e.g. by van Rood (21) and Dausset (5).

In healthy subjects leucocyte isoantibodies are only found as an effect of transfusion or pregnancy. The frequency of mothers with leucocyte anti-

bodies has been found to increase with the number of pregnancies (18, 19, 22). This indicates that the foetus like in Rh immunisation, can immunise the mother. A difference from Rh immunisation is, however, that these leucocyte antibodies, according to investigations by Jensen (11, 12) and Payne (18, 20), seem to produce leucopenia in the child only exceptionally. None of these authors could ascertain leucopenia even in cases with high antibody titres. Transplacental passage of antibodies was shown by Payne in one third of the cases. The number of leucocytes in this group of children did not differ from the number of leucocytes in a control group where the mothers lacked leucocyte antibodies.

Although cases have been described in which a relationship between granulocytopenia in the child and the presence of leucocyte antibodies in the mother seems very likely, there is nothing to prove that such a relation is a common phenomenon. Obviously several factors e.g. concerning properties of the antigens or antibodies pro-

duction of leucocytes in the infant etc may be involved. The case presented below deserves interest because the leucocyte antibodies present were established to be group specific and consisting of IgG globulin.

Case report

Boy born 8.11.1963. The mother was a 29 year old primipara blood group B Rh(+) earlier healthy, no blood transfusions. During the last trimester minimal oedema and a slightly raised blood pressure occurred. Delivery seven weeks before term. Birth weight 2360 g. Half an hour after delivery the child became cyanotic and showed signs of irregular breathing and was therefore admitted to the Pediatric Department. After 15 minutes in an environment of increased O_2 he was again in good condition. Blood examination 12 hours after birth showed absence of the neutrophilic peak present in normal newborns (total leucocytes 10900 per mm^3 of which 3100 polynuclear cells per mm^3). However the finding was not so striking that further study was considered necessary. On the fourth day the serum bilirubin value was found to be 24.8 mg per 100 ml.

The blood group of the child was O Ph(+) and the direct antiglobulin (Coombs) test was negative. No irregular erythrocyte antibodies were found in the mother's serum. An exchange transfusion was performed and again repeated two days later when the bilirubin concentration was 26.8 mg per 100 ml serum. The bilirubin values after that showed a downward slope. The patient's general condition was good and he gained weight steadily. On the sixteenth day the boy became apathetic and cried faintly. Neurological examination did not reveal any gross abnormalities. The spinal fluid was faintly yellow with a total protein value of 19.3 mg per 100 ml, cells 184 mononuclear, 6 polynuclear and 25 erythrocytes per 32 mm^3 . Bacterial cultures from spinal fluid and blood were negative. Penicillin (Daktacilin) was given as a pro-

phylaxis. A few days later the patient seemed to be free from symptoms. A blood examination on the eighteenth day revealed a neutrophil agranulocytosis. The total number of leucocytes was 7200 per mm^3 , non segmented granulocytes 10%, polynuclear cells 0%, eosinophils 110%, lymphocytes 770%, monocytes 10.3% and plasma cells 0.5%. Thrombocytes numbered 260 000 per mm^3 . A bone marrow specimen taken on December 1st showed a picture of agranulocytosis with almost complete "arrest" on the myelocyte stage. The erythrocyte precursors were somewhat sparse but otherwise normal. Megakaryocytes were few in number.

The child's condition remained quite satisfactory there were no clinical signs of infection and he showed good increase in weight. The blood picture was improved (Fig 1). Yet even after seven months there was a granulocytopenia (leucocytes 11 600 per mm^3 , segmented granulocytes 13.3%, lymphocytes 80.5% and monocytes 4.2%).

Serological examination

Serum samples from the mother were taken on December 8th i.e. four weeks after delivery. Using the agglutination technique according to Dausset (4) leucocyte antibodies active against leucocytes from the father, from the child and from twelve out of 30 O Rh(+) blood donors chosen at random were found in the serum. The father belonged to blood group B Rh(+) In order to test the mother's serum for antibodies to the father's leucocytes the mother's serum was first absorbed with erythrocytes from a B person whose leucocytes gave a negative reaction. The mother's antibody titre against the father's leucocytes was 1:8 against the child's 1:32 against three of the blood donors 1:8—32.

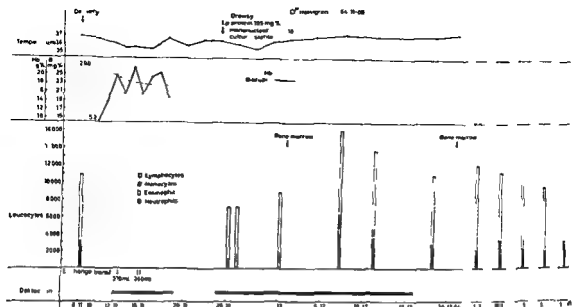


Fig 1 Course of the disease including blood examination from birth until twelve months of age

and against nine of the blood donors 1 2—8 Tests from the remaining eighteen blood donors gave negative reactions The examination was performed with defibrinated leucocytes

The results indicated the possibility of a relatively simple group specificity and therefore the serum was sent to the WHO Reference Laboratory for leucocyte grouping in Leiden for further study (Director J J van Rood MD) With a panel consisting of nine 8a positive and six 8i negative leucocyte samples it appeared that the antibodies in the serum recognized an antigen which showed a significant correlation with the antigen 8i (23) It was concluded that in all probability the serum had anti 8i specificity

Immunoglobulin examination

Serum was fractionated by gel filtration on a Sephadex G 200 column according to an earlier described technique (13) The fractions were concentrated and tested partly with the agglutination technique and partly with the antiglobulin consumption technique (9) As is shown in Fig 2 antibody activity was obtained only in the fractions corresponding to the

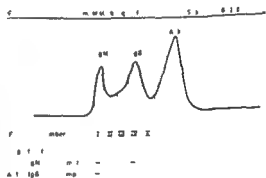


Fig 2 Fractionation and antiglobulin consumption experiment showing that the leucocyte antibodies of serum from the mother tested with leucocytes from the father consisted of IgG globulin

according to an earlier described technique (13) The fractions were concentrated and tested partly with the agglutination technique and partly with the antiglobulin consumption technique (9) As is shown in Fig 2 antibody activity was obtained only in the fractions corresponding to the

second main peak. This contains the main part of IgG and part of the IgA globulin in serum. The antioglobulin consumption test with a system specific to IgG globulin gave a positive reaction. The presence of antibodies of IgG type was therefore established but no antibodies of IgM type were demonstrated.

Discussion

The child described above was prematurely born. According to Jensen (11) there is an increased frequency of premature births in mothers with leucocyte antibodies. Increasing jaundice during the first few days of life necessitated two exchange transfusions. However no red cell antibodies were demonstrated and no ABO incompatibility between mother and child was present. The experience from exchange blood transfusions in cases of Rh erythroblastosis indicates that the exchanges as such do not give granulocytopenia.

The fact that maternal leucocyte antibodies relatively often appear during pregnancy as the result of isoimmunisation whereas leucopenia is an unusual phenomenon in the offspring necessitates caution in any conclusions of a true causal relationship between the two findings. However in the case here described there might be some basis for such a relationship since the antibodies were of IgG type giving a strong reaction with the child's leucocytes. It is well known that the IgG globulin passes the placenta. There might be several plausible explanations

why granulocytopenia in the infant is rare in spite of maternal leucocyte antibodies.

1 Probably the amount of leucocyte antibodies and their physicochemical characteristics are of importance (5). However the technique for leucocyte agglutination presently available is not quite ideal for quantitative determinations (5, 6). Therefore it may be difficult to draw valid conclusions from the antibody titres.

2 The antigen against which the antibodies are directed may be present in a number of cells in the organism which can contribute to a quick absorption and breaking down of the antibodies. Thereby the amount of antibodies bound to the leucocytes can be so low that the cells escape damage. Certain antigens are common to granulocytes, lymphocytes, thrombocytes and probably also to other cell types in the organism. Other antigens seem to be more specifically localised to a certain cell type (6). It might therefore be possible that a leucopenia could be developed by antibodies directed against antigens which exist almost exclusively in granulocytes but which are absent in most other cell types. Lalezari has recently presented evidence that the leucocyte antibodies active in his cases were pure anti-granulocyte antibodies (14). In our case the antibodies had the specificity anti-8a. At the recent workshop on leucocyte grouping in Leiden it appeared that the 8a antigen is if not identical closely linked to Mac-PIGLy^{Br} LA₂ and group 2 of Serizaki (J. J. van Rood

personal communication) and thus active not only to granulocytes (6). Further experience is required to determine if neonatal granulocytopenia is related to antibodies against only certain leucocyte antigens.

3 Another possibility could be that the elimination of the child's leucocytes from the circulation which might be caused by the antibodies is compensated by an already existing extra vascular reserve (3, 20) or by an increased new formation. Shulman (25) has shown that, after an injection of antibodies against leucocyte thrombocyte antigens, both cell types initially may decrease, then the granulocytes are rapidly restored whereas the number of thrombocytes increases more slowly. The leucocyte antibody effect may be potentiated in certain cases assuming that the leucocyte production reserve of the patient is unusually low. In our case the somewhat low granulocyte values even over a protracted period indicate that such a mechanism might have been active.

4 A fourth possibility is that leucopenia and maternal antibodies are unrelated phenomena. This possibility cannot be excluded in the present case. Unfortunately the first sample for leucocyte antibody determination was drawn four weeks after birth. Thus, it could not be proven that the maternal antibodies were produced before delivery.

Summary

A case of granulocytopenia occurring in a newborn infant is described. The

mother's serum contained leucocyte antibodies active against the child's and the father's leucocytes. The antibodies belonged to serological type anti 8a and consisted of IgG globulin. The relation between the granulocytopenia and the serological findings is discussed.

Acknowledgement

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Table 1 Cancer death rates 1960—1962 (Age adjusted)

Male			Female		
Order	Site	Rate ¹	Order	Site	Rate ¹
1	Lung	33.4	1	Breast	22.2
2	Colon rectum	18.8	2	Colon rectum	17.0
3	Prostate	13.2	3	Uterus	13.2
4	Stomach	11.8	4	Ovary	7.6
5	Pancreas	8.2	5	Stomach	5.9
6	Leukemia	7.5	6	Leukemia	4.8
7	Esophagus	3.8	7	Lung	3.0
8	Kidney	3.2	8	Pancreas	4.8

¹ Rate per 100 000 population

Source: American Cancer Society

dures potentially capable of preventing the development of leukemia in man have not yet been brought forward although there are indications that certain types of leukemia in mice may be partially protected against after appropriate vaccination as was shown by Friend (8) in 1959.

In the United States in children under 15 years of age leukemia accounts for about 35 per cent of all neoplastic disease. In the population as a whole (see table 1) leukemia ranks in about sixth place among the various forms of neoplastic disease and the age adjusted mortality rate for leukemia is about 6 per 100 000 population per year. This should be compared with an age adjusted mortality rate of approximately 125 per 100 000 population per year for all types of cancer in the United States. Thus leukemia accounts for about 5 per cent of all deaths from cancer in the United States or about 15 000 deaths per year.

The problem is both large and unsolved and much more knowledge is needed than has so far been obtained.

On several grounds it seems appropriate to suggest that the greatest defect in current knowledge of leukemia in man has to do with causal factors particularly the primary incitants. The importance of understanding the nature and the relative significance of causal factors needs no emphasis among those who are familiar with the remarkable record of the development of effective control measures for other major diseases which has been accomplished during the last 40 years. The control of most bacterial diseases with antimicrobial agents vaccines or toxoids of many viral diseases with viral vaccines and of several deficiency diseases with vitamins or hormones provides abundant evidence of the crucial role that knowledge of causal factors may play in the development of effective controls.

In the light of what has been learned since 1951 about the primary incitants of leukemia in numerous species of animals especially mice chickens rats and hamsters as well as cats and possibly cattle it is not surprising that many investigators tend to lean toward the hypothesis that leukemia in man may also be associated with certain viruses as yet unidentified. This theory has received some indirect support as a result of the finding by Lieberman and Kaplan (12) in 1959 that leukemia induced by irradiation of mice could in some instances be transmitted by cell free filtrates and the more recent finding that certain

Leukemia in man and Mouse

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To appreciate the significance and importance of leukemia in man, it is necessary only to recall that it continues to occur throughout the world's population and still causes severe illness and large loss of life, particularly during early years. In man its cause or causes are yet to be decisively demonstrated despite the fact that some 57 years have passed since Wilhelm Ellermann and Oluf Bing showed in 1908 that a form of leukemia of chickens could be transmitted by cell free filtrates and that some 14 years have gone by since Ludwik Gross demonstrated in 1951 that leukemia of mice was induced by a virus.

Its diagnosis in man is rarely accomplished until the disease is well advanced and has resulted in definite and often widespread pathological alterations which require the specific microscopic skills of the pathologist or hematologist for decisive recognition. Except for microscopy there are still no reliable or unequivocal laboratory procedures that serve as useful aids to diagnosis in patients. In contrast, it is now feasible to identify and

even to classify directly by *in vitro* immunologic procedures leukemic cells of several types from various species of animals particularly mice.

The treatment of patients even by the most modern procedures, as initiated in 1948, employing combined or sequential chemotherapeutic agents including steroids, or radiation over long periods of time, has been of value in achieving remissions and extending the median time of survival from about four months to approximately 13 months as has been determined at the Memorial Hospital but only rarely leads to long term remissions lasting as long as five years.

At the International Congress of Hematology in 1964 Burchenal (4) reported that a total of 93 patients with acute leukemia were then known to have survived five years or more after diagnosis. More recently he has stated that the number of long term survivors has increased to about 104. The factors responsible for such long survivals are not yet known and apparently are not correlated with one or another treatment program. Proce

More recently the presence of similar particles has been reported in myeloid leukemia and certain other types of lymphoma. During the past year several reports (15, 16, 2) recorded the finding of such particles in centrifugally concentrated sediments from leukemic plasma. It has been held that these virus-like particles are approximately similar in size and form to the mouse and chicken leukemia viruses but it is important to emphasize that no evidence for any biological activity has been presented and that several other workers have not obtained confirmatory results with comparable specimens.

In view of the uncertainties regarding the nature of such particles, the differences in their sizes and forms, and most importantly the fact that no biological activity has been reported, it seems premature to suggest that they represent viruses. Should they be found to satisfy all of the criteria required for the identification of viruses, it would still be necessary to determine what relation, if any, they may have to leukemia in man.

It should be emphasized that the finding of viruses in specimens from patients, especially those with neoplastic disease, does not of itself provide evidence for a relationship between the agent and the disease. Common viral agents or those that are simply "passengers" rather than "drivers" are both common and well known particularly in the respiratory tract (80 known viral types) and in the intestinal tract (60 known viral types) of human beings. In patients with se-

vere neoplastic disease such as leukemia it would not be surprising if such agents move about more readily than in normal persons and come to lodge in or even to infect neoplastic tissue.

During 1964 several workers in the United Kingdom and the United States reported the isolation of virus-like agents with definite biological activity from patients with leukemia (13) or the malignant lymphoma (6) of Africa, which has been suggested to represent a local counterpart of leukemia (5). In most instances these agents were recovered by the use of human cells in culture. There is considerable uncertainty about the nature of some of these agents and they are still under active investigation. Some have been found to be either mycoplasma, i.e. pleuropneumonia-like organisms, or associated with the presence of mycoplasma organisms which all too frequently are encountered in mammalian cell cultures that are maintained in series. One agent appears to have been identified as a reovirus (1).

Although it seems probable that certain of these agents were present in the specimens from which they were recovered, none has yet been shown to be oncogenic in animals or clearly to transform cells in culture. In contrast, the recognition of each agent has depended on its potentiality to produce cytopathic effects in cultured cells. Whether they should be considered as "passengers" or associated with leukemia or malignant lymphoma still remains uncertain. In a few instances (13, 6) tests for the presence of anti-

leukemogenic chemicals such as dimethylbenzanthracene appear also to activate latent or dormant leukemia viruses present in apparently normal host animals (17)

Among the three categories of primary incitants that have been found to be capable of inducing neoplastic disease in animals, i.e., ionizing radiation, oncogenic viruses, and carcinogenic chemical compounds, extensive studies relating to two or possibly bearing on leukemia in man have been carried out

As a result of the continuing investigations of the Atomic Bomb Casualty Commission in Japan, there appears to be good evidence that a single very intense exposure to ionizing radiation ultimately led to an increased incidence of leukemia among the survivors and that there was a direct relationship between the large quantities of radiation received and the frequency of leukemia induction (3). It needs to be emphasized however that such very large amounts of radiation are not encountered under ordinary circumstances and that extrapolation of the data to background levels of radiation exposure may not be justified. Nonetheless there remains an important question regarding the contribution, if any, of continuing exposure to so called background radiation to the incidence of leukemia in man. The fact that acute leukemia is more common among children under 15 years of age than among adults in the same environment is enough to cast considerable doubt on the theory that natural exposure to ionizing radiation

plays a significant role as a primary incitant. In addition, Dr. Leonard Hamilton (11) has kindly provided computations based on currently acceptable hypotheses which suggest that background radiation during the normal life span would not be expected to account for more than about 3 per cent of the natural incidence of leukemia in man.

Attempts to demonstrate viruses associated with leukemia in man have been reported frequently during the past 30 years and numerous intensive studies were stimulated by Gross's discovery of the first mouse leukemia virus in 1951 (9). Despite several recent suggestive reports, decisive evidence for an association between leukemia in man and viruses is still lacking. Innumerable techniques and many different species of animals have been employed in such studies so far without yielding unequivocal results. The use of newborn or thymectomized animals with deficient immune mechanisms as well as the employment of modern cell culture procedures, which have included various human cell types, have not been rewarding and leukemia viruses of man if in fact they exist remain to be found.

Several reports of the presence of virus like particles in the tissues or blood of patients with leukemia have appeared recently. Beginning in about 1959 virus like particles have occasionally been found by Dmochowski and others with the electron microscope in the lymph nodes of patients with acute lymphatic leukemia (7).

Table II Forms of leukemia and lymphomas induced in mice and rats with mouse leukemia virus type A

LEUKEMIA	LYMPHOMAS
Lymphatic Leukemia	Lymphosarcomas
a) aleukemic	a) local lesion
b) leukemic	b) generalized
Stem cell Leukemia	Reticulum cell sarcoma
Myelogenous Leukemia	Hodgkin's like lesions
a) undifferentiated	
b) well differentiated	
Chloro Leukemia	
Erythroblastic Leukemia (atypical)	
Monocytic like Leukemia	
Gross, L. <i>Acta Haemat (Basel)</i> 32 44 1964	

in mice as well as a number in hamsters rats guinea pigs and rabbits

So far as is presently known the mouse leukemia viruses like those of the chicken leukosis sarcoma complex are of the RNA type range in diameter from about 70 to 110 m μ but are of variable size and shape and tend to be either sensitive. Infective RNA preparations have been obtained from tissues infected with the Moloney and Graffi viruses but similar procedures have failed to yield infective RNA from tissues infected with the Gross and Friend viruses

These viruses are thought to multiply in the cytoplasm but do not become fully mature until they reach the cell membrane which appears to take part in the completion of the particle. The mature virus particle leaves the cell by a process called budding which does not usually lead to lysis or degeneration of the cell

The majority of mouse leukemia viruses are also pathogenic for rats and the Moloney virus is pathogenic for hamsters. Newborn animals are the most susceptible presumably because of the immaturity of their immune responses and are preferred in studies on the recovery of leukemia viruses particularly with spontaneous chemically or γ radiation induced leukemia. Some of these agents are pathogenic for young animals and certain of them e.g. the Friend virus is also pathogenic for adult animals

Depending on their genetic make up mouse strains may vary widely in their susceptibility to leukemia viruses. Other host factors such as hormonal balance and nutritional states may also affect susceptibility. The chromosome number and karyotype of virus induced mouse leukemia cells appear not to differ markedly from those of control cells

In contrast to oncogenic viruses of the DNA type such as polyoma and Simian virus 40 the RNA containing leukemia viruses multiply readily in neoplastic tissues and considerable numbers of mature infective particles are produced. This has facilitated their demonstration by both biological and physical procedures. Despite the concentrations that can be obtained in purified preparations the specific antigenicity of leukemia viruses as such tends to be relatively weak and is particularly poor with Gross virus. Specific antiviral sera can be obtained but they are not often of high titer and may give irregular results in cross

bodies in the serum of patients have yielded results suggesting that antibodies against the agents were present in some patients but not in control sera. Such evidence, even if confirmed, would not itself establish an association between the agents and the disease, although it would indicate that an immune response had occurred, presumably as a result of an infective episode. Unfortunately, at the present time, it is much easier to show that a given virus or other infective agent is not causally associated with leukemia in man than it is to demonstrate that it may serve as a primary incitant of the disease.

In contrast to the inadequacy of current knowledge of the etiology of leukemia in man, much is known of the etiology of leukemia in the mouse. Because the disease in the mouse may serve as a useful and stimulating model of the disease in man, the subject merits a brief review.

Many workers now incline to the view that mouse leukemia regardless of morphological type or strain of mice is, in fact, a viral disease. Relative to naturally occurring neoplastic disease in animals, mouse leukemia and the chicken leukosis sarcoma complex appear to be more fully understood than any others. Some 14 leukemogenic virus strains have been recovered from mice since the first mouse leukemia virus was discovered by Gross in 1951. It is doubtful that these are all definitely different viruses and it seems more probable that several may represent variants of one stem virus. On immunological

analysis, for example, it appears that three of the viruses, i.e., the Friend Moloney, and Rauscher agents, form a cross reacting group and are closely related to one another (14). They are, however, not related immunologically to Gross virus which, it appears, should now be regarded as the mouse virus that typifies naturally occurring mouse leukemogenic viruses.

The Gross virus leads to the appearance of a cell antigen which is distinct from other known antigens found in mouse leukemia. This antigen appears to characterize all leukemias, regardless of morphological type, induced by the Gross virus as well as many forms of spontaneous mouse leukemia and those induced by chemical compounds or by X-radiation. In contrast, cell antigens of the Friend Moloney, Rauscher type have not been found in spontaneous chemically or X-radiation induced leukemia. They appear only in leukemias induced by inoculation of these viruses (14).

On morphological grounds many different forms of leukemia or lymphoma may be induced in mice or rats by the Gross virus. Gross (10) recently compiled a list (see table II) of some 12 forms of neoplastic disease that have been induced by the Gross mouse leukemia virus which he now designates as Type A. The variety of morphological types of neoplastic disease which may result from infection with a single virus is not unique and, in fact, has been exceeded by the even more striking potentialities of polyoma virus which has induced more than 20 different types of solid tumors.

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neutralization tests. This has made immunological studies on the agents themselves difficult to evaluate.

Inactivated leukemia virus vaccines have been prepared and, with certain agents, e.g., the Friend virus, have been shown to be moderately effective in producing immunity against leukemia induced by the same virus.

Despite the weak antigenicity of leukemia viruses, the leukemic cells of animals infected with such viruses contain new antigens which make it feasible to distinguish certain immunological types of mouse leukemia by *in vitro* procedures. The cytotoxic test which has been intensively studied by Old and Boyse (14), has the advantages of simplicity and speed when appropriate immune sera are available. Immune sera may be produced either by growth and rejection of transplanted isogenic leukemia or infection with a leukemogenic virus in adult mice. In the presence of guinea pig complement such sera are cytotoxic for leukemia cells which possess new antigens homologous with those of the leukemia used for immunization or the leukemia induced by the virus used for immunization. Because of the use of isogenic leukemic tissue there is no immune response to the transplanted cell antigens other than those new antigens which characterize the leukemic cells.

Old and Boyse (14) have found five distinct cell antigens demonstrable by the cytotoxic test in mouse leukemias. Three of the antigens are associated with known leukemogenic viruses, i.e.

1) Gross virus, 2) Friend, Moloney, and Rauscher viruses, and 3) mammary tumor agent. Two other antigens are now known to be associated with the presence of leukemogenic viruses and may take their origin from the genetic material of the leukemia cell. Soluble circulating antigens, separable from infective virus and with the same specificity as the cell antigens, have been demonstrated in the plasma of mice with leukemia induced by the Gross, Friend-Moloney, or Rauscher viruses.

It seems possible and certainly is to be hoped that these solid advances in knowledge of the immunology of leukemia in the mouse may have applications to leukemia in man. Although there are serious genetic barriers which greatly impede comparable studies in human beings, it appears that they may not be insurmountable and that a search for analogous cell antigens in leukemia in man may be feasible.

As a concluding comment it seems appropriate to emphasize that there are many close similarities between leukemia of the mouse and leukemia of man. In the light of what has been learned of the etiology of mouse leukemia, it would be difficult to defend the view that viruses are improbable candidates for a role as primary inductants of human leukemia. It is to be expected that further studies will ultimately provide decisive answers which will either support or disprove the hypothesis that viruses are associated with leukemia in man.

phytohaemagglutinin (PHA) and for 72 hours with PHA

1 Two male patients with similar alteration in karyotype (JH and T)

Clinically the two patients were unlike. Neither had received X-ray therapy both had been treated with busulphan. T had been treated since 1951 his clinical condition was well controlled initially with arsenic and urethane later by intermittent courses of busulphan. In February 1965 when the chromosomes were examined the patient felt well but the liver and the spleen were enlarged and blast cells had appeared in the blood. The terminal illness was rapid and the patient died aged 61 on March 5th 1965 after 14 years in the chronic stage.

JH in contrast had a longer terminal illness following satisfactory control for 2 years by continuous busulphan therapy. Blast cells appeared in the peripheral blood and the haemoglobin concentration began to fall. Mercaptopurine was ineffective. Four months later (January 1965) he developed jaundice, fever and a perineal abscess. He was deeply pigmented and had lost weight. The haemoglobin level continued to fall in spite of blood transfusions. He died on March 21st 1965 aged 51.

In Romanowsky stained bone marrow films in both cases the majority of the cells were blast cells.

The karyotypes were studied in cells grown in blood cultures. In T 13 of the 14 cells examined contained the Ph¹ chromosome only one of the Ph¹ positive cells was normal in respect of the remaining chromosomes. Twelve cells contained two extra chromosomes in the C group and 10 of them lacked one chromosome in the E group.

In JH 13 of the 15 cells examined contained the Ph¹ chromosome. All 13

also contained extra C group chromosomes one in two cells two in nine cells and four in one cell. Eleven lacked an E group chromosome. The most deranged cell had four extra C group chromosomes one extra G and lacked two E group chromosomes. Thus the chromosome complement varied in the cells in both cases from 45 to 49.

The similarity in chromosome rearrangement in these two cases was not associated with any similarity in the clinical evolution of the terminal blast cell changes.

Pedersen reported two cases of this type of change in karyotype in young men (6). In one case the abnormal cell line appeared 2½ months before death and in the other 3½ years after the diagnosis of CGL and 6 months before death. The karyotype changes in these four cases cannot be assumed to be identical. The morphological identification of individual members of the same group of chromosomes is not possible and numbers 17 and 18 of the E group cannot be identified with certainty.

2 Three males with an XO chromosomal complement in the bone marrow cells

Two of these patients, EI (case no 22) (7) and DJ did not show any additional karyotype changes in the terminal stages.

EI who had leucocytes was 28 when CGL was diagnosed in 1951. After an initial course of radiotherapy in the spleen the disease remained well controlled by continuous busulphan therapy. Mercaptopurine was substituted towards the end of 1964 because of anorexia, weight loss, deep pigmentation and fall in the blood pressure and the haemoglobin concentration. In January 1965 blast cells

Chromosome Changes in the Terminal Stages of Chronic Granulocytic Leukaemia

By S D LAWLER and D A G GALTON

The bone marrow cells of classical chronic granulocytic leukaemia (CGL) almost always contain a characteristic abnormal small acrocentric ('Philadelphia', Ph^1) chromosome (no 21, Denver nomenclature, 5, 2). Some patients who have Ph^1 -positive cells are not, however, clinically or haematologically typical though the Ph^1 -negative cases are far more heterogeneous (4).

The Ph^1 chromosome is confined to the bone marrow cells, the cells in skin and lymphocyte cultures are normal. In the polyploid cells (probably megakaryocytes) of bone marrow the number of Ph^1 chromosomes corresponds to the ploidy. The evidence relating to erythrocyte precursors is indirect. In a typical case more than 90% of the bone marrow cells are Ph^1 positive whatever the ratio between erythropoietic and granulopoietic cells.

In the chronic phase of the disease apart from the presence of the Ph^1

chromosome, the karyotype is usually normal. The bone marrow cells of a few affected males not necessarily clinically similar, are $XO\ Ph^1$ positive. $XX\ Ph^1$ positive cells may also be present (1, 3, 7). The cells in lymphocyte and fibroblast cultures, however, have been found to be normal XX and some of these patients have had male children. CGL has also been described in a sex chromosome mosaic of the XX/XY type (3).

Sometimes, additional chromosomal anomalies appear in the terminal stages of the disease. These changes tend to be unique for each patient. We now examine the clinical circumstances in which these changes have arisen.

Some of our 8 patients (all males) studied during the chronic phase were previously described (7). The absence of females is almost certainly fortuitous. Unless otherwise stated the chromosomes in bone marrow were examined by a modified "direct" technique (8). Blood cultures were usually made for 24, 48 and 72 hours without

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chromosome was not the first additional abnormality, because only one was present in the earliest stage of the blast cell crisis. The original population was eventually replaced by even more abnormal cells. The treatment with dibromomannitol destroyed many blast cells but after treatment cells appeared even more deranged than those present before. The patient died on July 30th 1965.

Thus the clinical course during the chronic phase, and the events of the terminal illness of these three male patients with an initially similar Ph^1 positive XO karyotype were very different. In two cases the chronic phase was benign and well controlled for several years but in one of them the terminal phase was acute and associated with the appearance of several abnormal karyotypes showing evidence of variation and selection in the population of blast cells while in the other the terminal illness was protracted and not associated with any change in karyotype. The third patient had a short poorly controlled chronic phase, a long terminal illness with blast cell increase in the blood and bone marrow but no change in the original karyotype.

3 Extremely rapid terminal changes accompanied by karyotype changes not involving the entire cell population

H.O. a Jamaican was 59 when CG1 was diagnosed in 1963. There was gross splenic enlargement and generalised but minimal lymphadenopathy. The haematological picture was that of classical CG1. The karyotype

studied in a blood culture was Ph^1 positive and showed no other abnormalities. He responded well to busulphan but it was difficult to stabilise the leucocyte count.

From mid December the haemoglobin concentration hitherto well maintained fell rapidly and on January 12th 1965 50 per cent of the bone marrow cells were blasts only occasional blast cells were seen in blood films. The karyotypes of 13 bone marrow cells (January 12th 1965) all contained the Ph^1 chromosome. Six had no other abnormalities whilst seven lacked two C group chromosomes and had one extra D group chromosome. One of these seven cells also had an extra C-group chromosome and another two extra E group ones.

It is probable that the cells with the abnormal karyotypes were the blast cells seen in the Romanowsky stained films, the cells with the original karyotypes being the residual cells of the chronic phase. The terminal changes were extremely rapid, the patient died on February 16th 1965.

4 Two patients who showed the development of successful clones of abnormal cells in the terminal stages

J.D. was 40 when CG1 was diagnosed in April 1963. He responded well to busulphan until June 1964. He never received radiotherapy. A bone marrow preparation (July 1964) showed only Ph^1 positive otherwise normal cells. The patient then had a large liver and spleen and pericardial and pleural effusions. His condition deteriorated and he died on October 4th 1964.

In a blood culture (October 3rd 1964) the most frequent chromosome

appeared in the blood. A chest infection in March 1963 responded to antibiotics but he became weaker and developed signs of a splenic lesion. A bone marrow preparation in January 1963 produced few dividing cells all were $\lambda O Ph^1$ positive. Romanowsky stained films showed granulocytic hyperplasia without in excess of blast cells.

D J was 43 when CGI was diagnosed in 1963. He had previously had malaria with enlargement of the spleen which however was not felt in 1963. The leucocyte count was 160 000 per cumm of which 320 were blasts. He responded to splenic irradiation but relapsed rapidly. Little improvement was subsequently obtained with busulphan and prednisone therapy. He had recurrent attacks of gout. He remained anaemic and died in December 1964 18 months after the diagnosis had been made. Satisfactory clinical and haematological control not having been achieved.

Blast cells up to 11 500 per cumm were present in the blood during the last 18 months of life. In Romanowsky stained films large and small blasts with distinctive morphological characteristics were found. Only the large blasts fell in number during busulphan therapy. This dual population of cells was not reflected in karyotype differences. The dividing cells were few in number in the various preparations made but only $\lambda O Ph^1$ positive cells were ever found in blood cultures or bone marrow.

D C (no 23) (7) was 27 when CGL was diagnosed in 1957. The disease showed the classical clinical and haematological features of CGI and was well controlled for 7 1/2 years by continuous busulphan therapy. He received no radiation therapy but mannitol mustine was given for a month in 1957. During 1962 the bone marrow cells analysed all contained the Ph^1 chromosome and lacked the λ chromosome. The blood lymphocytes and the skin fibroblasts all had a normal male karyotype and were Ph^1 negative. Serial examinations of the chromosomes were made during the terminal stages. The changes were manifold but all the cells lacked a G group chromosome (presumably λ) and contained at least one Ph^1 chromosome. The additional

changes were progressive and appeared to be superimposed on the cell line of the chronic phase.

In February 1963 the haemoglobin concentration began to fall. On May 20th 1963 the marrow contained 84 per cent of blast cells and rearrangements were already present in the chromosomes in both blood and bone marrow. The karyotype of 4 of the 21 cells analysed was the same as that found in the chronic phase ($\lambda O Ph^1$ positive). The remaining 17 cells showed additions of the D or F group and loss of L group chromosomes. The chromosome number varied from 44 to 49.

On May 24th 1963 busulphan was replaced by dibromomannitol, administered until June 10th 1963. The blast cell count fell rapidly and in a blood culture (June 10th) only severely damaged chromosomes were found.

On June 24th 1963 every $\lambda O Ph^1$ positive cell in a blood culture now showed additional changes, five of the 10 cells analysed had two Ph^1 chromosomes. Of the four cells with 48 chromosomes, two had the same chromosome complement including two Ph^1 chromosomes and the two with 49 chromosomes resembled them but in addition had an extra C group chromosome. The remaining four cells had 45 chromosomes. In a fourth culture (July 8th 1963) all the cells with more than 46 chromosomes now had two Ph^1 chromosomes. Four cells had 48 chromosomes that were identical with one another but differed from the cells in the previous culture having 48 chromosomes in having an extra C group chromosome instead of an F. Thus a series of rearrangements were present basically similar but varying amongst themselves. Possibly a cell line with 48 chromosomes was beginning to dominate. The appearance of a second Ph^1

chromosome was not the first additional abnormality, because only one was present in the earliest stage of the blast cell crisis. The original population was eventually replaced by even more abnormal cells. The treatment with dibromomannitol destroyed many blast cells but after treatment cells appeared even more deranged than those present before. The patient died on July 30th 1965.

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Table I A J Blood Counts and Chromosome Studies

Date	Total WBC/ cu mm	% Blasts in PB	Preparation	Chromosome Number								No of cells
				46	47	48	49	50	51	52		
29 7 63	77 000	15 %	PB 24 hrs	1	0	1	10	12	1	0	23	
			PB 26—28 hrs	2	2	1	4	22	1	0	37	
13 8 63	8 000	5 %	PB 40 hrs	4	0	2	10	5	1	0	28	
15 8 63	7 600	5 %	BM Dir	0	0	2	2	4	0	1	9	
			BM 24 hrs	2	0	2	3	16	1	0	24	
19 9 63	5 200	7 %	PB 24 hrs	1	0	1	3	12	1	0	18	
WBC leucocyte count PB peripheral blood BM bone marrow												

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number was 48 (23 out of 50 cells). Ten of the 23 cells with 48 chromosomes that were analysed or karyotyped had two Ph^1 chromosomes and an extra chromosome in the C group. A few cells typical of the chronic phase remained. Most of the cells with more than 46 chromosomes had two Ph^1 chromosomes. This acquisition of a second Ph^1 chromosome in the terminal stages was described by Court Brown (3). In addition there is evidence here for the emergence of a stable clone of cells.

A J, a West Indian, was 27 in 1962 when the diagnosis of CGL was made. The spleen was irradiated following a transfusion and a short course of busulphan. The response was poor and busulphan therapy was resumed. The level of haemoglobin well sustained until May 1963 then fell; the spleen enlarged rapidly and the blast cell count in the blood rose steeply. From July 25th 1963 he received blood transfusions, intravenous injections of Mannitol, Myleran and splenic irradiation with little benefit and died on October 2nd 1963. The disease was never controlled by radiotherapy or busulphan and the terminal blast cell phase began less than one year from the time of diagnosis. At presentation the blood and bone marrow findings were

those of classical CGL. A blood culture (November 15th 1962) showed Ph^1 positive normal male cells.

Serial chromosome studies were made during the terminal illness. Table I shows the chromosome counts obtained, the leucocyte counts and the percentage of blast cells. The cell lines with 49 and 50 chromosomes maintained a constant karyotype throughout the illness in contrast to the progressive and variable changes observed in D.C. The chromosome constitution of the majority of the cells in each culture with 49 chromosomes was 46 B4 C16 D6 E6 F6 G5 (1 Ph^1 positive chromosome); that of the cells with 50 chromosomes was identical except for the presence of a second Ph^1 chromosome (Fig 1). Varying the cultural conditions scarcely altered the proportion of cells with 49 and 50 chromosomes but the 40-hour blood culture contained a higher proportion of cells with 49 chromosomes than any other. Nor did the proportion change throughout the illness and only 5 cells out of the 136 observed had more than 50 chromosomes. One



Fig 1 karyotype of cell with 20 chromosomes from patient A J

of the cells with 21 chromosomes had the same analysis as that of the line of cells with 20 chromosomes with a second extra C group chromosome

In this case as in that of DC serral chromosome studies showed that abnormalities in addition to the presence of the Ph¹ chromosome appeared in the terminal blast cell phase. In DC the derangements were progressive and appeared to indicate instability in a cell population whereas the cells of A J showed the dominance of two stable and apparently related cell lines. These lines persisted over a period of 7 weeks in spite of changes in the blast cell count brought about by the treatment administered.

Comment

The terminal stage of CGL affords an opportunity of studying the accumulation of additional chromosomal abnormalities in a cell population in different patients already known to have a characteristic and constant abnormality.

All the secondary changes observed have in fact occurred in cells carrying the primary abnormality that is the Ph¹ chromosome. The changes have consisted in additions and losses in the normal chromosome complement rather than structural abnormalities and there has been a tendency to hyperdiploidy.

The earliest as well as the successive secondary changes varied both between patients and sometimes within the population of cells in an individual. This does not preclude the possibility that the terminal changes are initiated by mutation of an individual cell not reflected in karyotype abnormalities. Indeed in two cases the blast cell crisis was not associated with increasing aneuploidy but terminally only small numbers of dividing cells could be examined. In both cases the Y chromosome was absent from the marrow cells throughout the disease.

Similar additional abnormalities have recurred in different patients which perhaps suggests that they confer selective advantages to the cells carrying them. The tendency for certain cell lines to persist to dominate or to replace others indicates that an abnormality with survival value must also be stable.

The abnormalities observed could not be correlated with the duration of the chronic phase or with the rate of progression of the terminal phase.

Acknowledgements

We are indebted to the Consultant Staff of the Royal Marsden and Hammersmith Hospi-

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WBC leucocyte count, PB peripheral blood BM bone marrow

number was 48 (23 out of 50 cells). Ten of the 23 cells with 48 chromosomes that were analysed or karyotyped had two Ph¹ chromosomes and an extra chromosome in the C group. A few cells typical of the chronic phase remained. Most of the cells with more than 46 chromosomes had two Ph¹ chromosomes. This acquisition of a second Ph¹ chromosome in the terminal stages was described by Court Brown (3). In addition there is evidence here for the emergence of a stable clone of cells.

A J, a West Indian, was 27 in 1962 when the diagnosis of CGL was made. The spleen was irradiated following a transfusion and a short course of busulphan. The response was poor and busulphan therapy was resumed. The level of haemoglobin well sustained until May 1963 then fell. The spleen enlarged rapidly and the blast cell count in the blood rose steeply. From July 20th 1963 he received blood transfusions, intravenous injections of Mannitol Myleran and splenic irradiation with little benefit and died on October 2nd 1963. The disease was never controlled by radiotherapy or busulphan and the terminal blast cell phase began less than one year from the time of diagnosis. At presentation the blood and bone marrow findings were

those of classical CGL. A blood culture (November 15th 1962) showed Ph¹ positive normal male cells.

Serial chromosome studies were made during the terminal illness. Table I shows the chromosome counts obtained, the leucocyte counts and the percentage of blast cells. The cell lines with 49 and 50 chromosomes maintained a constant karyotype throughout the illness in contrast to the progressive and variable changes observed in DC. The chromosome constitution of the majority of the cells in each culture with 49 chromosomes was 46 B4 C16 D6 E6 F6 G5 (1 Ph¹ positive chromosome) that of the cells with 50 chromosomes was identical except for the presence of a second Ph¹ chromosome (Fig 1). Varying the cultural conditions scarcely altered the proportion of cells with 49 and 50 chromosomes but the 40 hour blood culture contained a higher proportion of cells with 49 chromosomes than any other. Nor did the proportion change throughout the illness and only 5 cells out of the 136 observed had more than 50 chromosomes. One

Is the Defective Reaction to Phytohaemagglutinin shown by the Lymphocytes from Lymphocytic Leukaemia Depending on their Innate Structure or on Plasmatic Characteristics?

By G. ASTALDI M.D. G. COSTA Ph.D. R. AMO M.D.
and N. DUATE Rcs Tech

Alterations in the composition of blood proteins are a rather common occurrence in disorders of the histolympathic system. About two decades ago J. Waldenström described a new syndrome characterized by the presence of an elevated amount of macroglobulins in the serum and of a "lymphoid" metaplasia in the hemopoietic organs (1). This syndrome has since been named *Waldenström's Macroglobulinemia*.

In recent years hypogammaglobulinaemia with impaired immunoresponse has been reported as a frequent feature of Chronic Lymphocytic Leukemia (CLL) (2). On the other hand it has been proved in different laboratories that peripheral blood lymphocytes from CLL patients give a low percentage of blasts when stimulated with Phytohaemagglutinin (PHA) in tissue culture (3-5). Among the lymphoid cells we obtained a percentage of blasts of $9 \pm 3\%$ in CLL after 72

hours of culture with PHA as compared with $68 \pm 5\%$ in healthy persons and of $69 \pm 6\%$ in chronic granulocytic leukaemia.

The defective response shown by CLL lymphocytes can be due either to the intimate biology of these cells (their age, their leukaemic condition, their immuno-incompetence and therefore their PHA-unresponsiveness) or to some inhibitory factors present in their plasma or in the culture system. In order to bring some light in this problem a series of experiments was undertaken by our group. In this paper we discuss the results concerning the observation that CLL plasma is capable of inhibiting the blastic development of lymphocytes from healthy persons when these lymphocytes are submitted to the PHA stimulation in tissue culture.

Material and methods

In these experiments the cultures were set up according to the technique

¹ Supported by The Blood Research Foundation, Washington D.C. USA

tals who have referred patients to us and we also thank Mrs I L Hansteen and Mrs P Dodd for assistance with the chromosome studies

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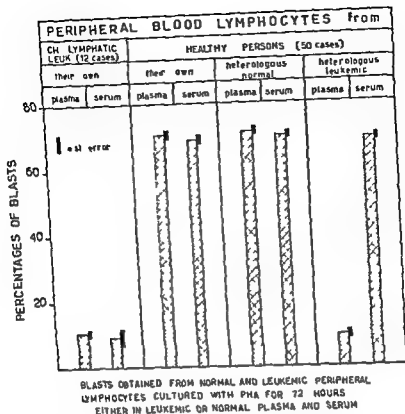


Fig 1

cytes were cultured with normal plasma. In other words, normal lymphocytes when cultured with CLL plasma clump like CLL lymphocytes.

d) finally, the lymphocytes from CLL patients cultivated in medium added to their own plasma and in medium added to their own serum developed a percentage of blasts of 10.0 ± 4.4 and 8.0 ± 6.3 respectively.

2 — The results of the experiment c) are summarized in the graphs of Fig. 2. It appears that peripheral blood lymphocytes from CLL patients gave very low percentages of blasts when cultured with plasma from healthy

persons and that the addition of plasma from healthy persons did not modify the usual PHA unresponsiveness of cultured CLL lymphocytes also when these lymphocytes were previously washed 1—4 times. In fact, the percentages of blasts obtained after 72 hours of tissue culture with PHA were on an average

a) 6.9 ± 2 when the lymphocytes from CLL were cultured without previously washing in medium added to plasma from healthy people and 3.3 ± 3.3 when the same CLL lymphocytes were added to the CLL plasma.

b) 7.4 ± 7.7 , 9.7 ± 7.2 , 9.4 ± 8.3 , $8.6 \pm$

described elsewhere (10), and the percentage of blasts among the lymphatic cells was determined after 72 hours of tissue culture. The investigation consisted of the following three experiments

a) *Lymphocytes from healthy people cultured in a medium containing PHA and plasma from CLL-patients* — Peripheral blood lymphocytes from 50 healthy persons were cultivated in NCTC 109 (Microb Ass) added to PHA (prepared by us in our Lab) (11) and divided in three lots. The first lot was added to autologous plasma (20 %), the second lot to heterologous normal plasma (20 %), and the third lot to plasma from 6 different CLL patients (20 %). At the same time the peripheral blood lymphocytes from these CLL patients were cultivated in the same NCTC 109 with PHA added to 20 % of their own plasma.

b) *Lymphocytes from healthy persons cultured in a medium containing PHA and serum from CLL-patients* — The details of this experiment were similar to those of experiment a) with the only difference that instead of plasma from CLL patients serum from the same 6 CLL patients was used.

c) *Lymphocytes from CLL-patients washed and then cultured in a medium containing PHA and plasma from healthy people* — Peripheral blood lymphocytes from each of 6 CLL patients were divided in two lots: the first lot was cultivated in NCTC 109 added to PHA and to heterologous normal plasma (20 %), the second lot was cultured in the same medium with

PHA and added to autologous (leukaemic) plasma (20 %) this was used as control. For each of these two lots, 5 series of culture flasks were prepared, in the first, second, third and fourth series the lymphocytes were cultivated after washing with pure culture medium 1, 2, 3 and 4 times, respectively, in the fifth series the lymphocytes were cultured without any previous washing.

Results

1 — The results of the experiments a) and b) are recorded in the graphs of Fig 1. It is clearly evident that the addition of plasma from CLL patients to cultures of lymphocytes from healthy persons inhibits the blastic development of these cells, whereas the addition of the same amount of serum from the same patients has no effect. In fact, blood lymphocytes from healthy people after 3 days of tissue culture with PHA developed the following percentages of blasts:

a) 71.5 ± 6.0 and 68.5 ± 7.9 , when added to their own plasma and to their own serum respectively.

b) 73.5 ± 3.5 and 70.5 ± 3.0 when added to a heterologous normal plasma and to a heterologous normal serum respectively.

c) only 10.0 ± 2.7 when added to plasma from CLL patients but 69.5 ± 2.0 when added to serum from the same CLL patients. Moreover these lymphocytes from healthy persons cultivated with CLL plasma appeared much more densely clumped together than when the same normal lympho-

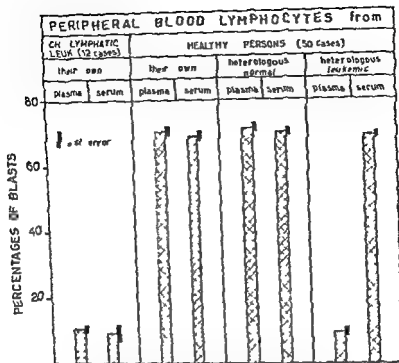


Fig 1

cytes were cultured with normal plasma. In other words normal lymphocytes when cultured with CLL plasma clump like CLL lymphocytes.

d) finally the lymphocytes from CLL patients cultivated in medium added to their own plasma and in medium added to their own serum developed a percentage of blasts of 10.0 ± 4.4 and 8.0 ± 6.3 respectively.

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a) 6.9 ± 0.2 when the lymphocytes from CLL were cultured without previously washing in medium added to plasma from healthy people and 5.3 ± 3.3 when the same CLL lymphocytes were added to the CLL plasma.

b) 7.4 ± 0.7 7.9 ± 7.2 9.4 ± 8.3 $8.6 \pm$

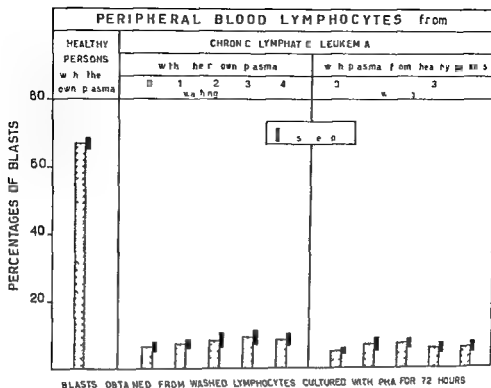


Fig 2

51 when the CLL lymphocytes were washed 1, 2, 3 and 4 times and then cultured with normal plasma and 7.0 ± 5.9 , 7.5 ± 5.5 , 7.7 ± 5.9 and 6.6 ± 4.8 when the same washed lymphocytes were cultured with their own plasma. In other words the number of blasts obtained in the experimental and in the control groups was of the same order of magnitude.

Discussion

The defective reaction of lymphocytes from CLL to PHA stimulus in tissue culture is a well documented phenomenon whereas the causes why the lymphocytes of these patients give so low percentages of blasts in culture with PHA are not yet definitely established.

Bernard et al (7) and Oppenheim et al (12) admit that in CLL there exists an inverse relation between the total leucocyte count in the peripheral blood and the number of blasts obtained in culture with PHA. On the other hand Elves et al (13) believe that only the lymphocytes from cases of CLL with hypogammaglobulinaemia give a low percentage of blasts in tissue culture with PHA whereas the lymphocytes from CLL patients with normal amount of gammaglobulin should give an almost normal percentage of blasts. In our experiments we have not observed any relationship between the total leucocyte count and the percentage of blasts obtained in culture nor between the gammaglobulin content and the blastic development of the lymphocytes of our patients. 9 of the 12 cases

of CLL reported showed around normal values of γ globulin and the percentages of blasts were low in all of them. Moreover it has been observed that lymphocytes from cord blood, as well as lymphocytes from hypogammaglobulinaemic and from agammaglobulinaemic patients give very high percentages of blasts in cultures with PHA (14). Thus we have been tempted to relate the defective reaction to PHA shown by CLL lymphocytes to the fact that these cells tend to clump in the culture flasks preventing in this way the surface action of PHA (10).

The defect of response to PHA shown by CLL peripheral blood lymphocytes in tissue culture could depend also on the possible existence of two clones of lymphocytes with different kinetics, different immunological competence, different sites or origin. In fact peripheral blood lymphocytes come from different sources: the majority of them from the mantle zones around the lymphoid germinal centers and from the medullary cords and pulp of lymph nodes and spleen whereas the remaining blood lymphocytes come from germinal centers, thymus cortex and bone marrow. The former seems to be the immuno-competent cells and the latter are believed to be the immuno-non-competent ones (15, 16). It is possible to hypothesize that in CLL a clone of lymphocytes prevails which comes from those sites (one or more) in which the lymphocytes are immuno-non-competent and that the defective reaction to PHA shown by the lymphocytes in CLL is just an exaggeration of a situation pre-

sent in healthy persons where also as it is well known some lymphocytes are PHA non responsive.

The results of our experiment a), reported above show very clearly that the plasma from CLL patients added to the cultures of peripheral blood lymphocytes from healthy people inhibits the blastic development of these cells. This observation is in contrast with those made by Schreck (4), Robbins (14) and Oppenheim (12) who reported that the addition of plasma from CLL patients to the cultures of lymphocytes from healthy persons do not inhibit the blastic development of these cells. On the other hand differences in technique and in the amount of CLL plasma added to the culture medium existing between our experiment and those of the above mentioned authors make it difficult to compare the results.

The observation that lymphocytes from healthy persons tend to clump when cultivated in a medium containing plasma from CLL suggests that the defective response to PHA shown by normal lymphocytes in these conditions could be due to the fact that PHA cannot reach the cell surface and initiate the blastic development just as it happens for CLL lymphocytes. On the contrary, the third part of our investigation shows that the deficient responsiveness of CLL lymphocytes to PHA in tissue culture could primarily be due to an intimate cellular defect rather than to the action of the inhibiting CLL plasma. In fact CLL lymphocytes previously washed several times and then cultured with PHA in

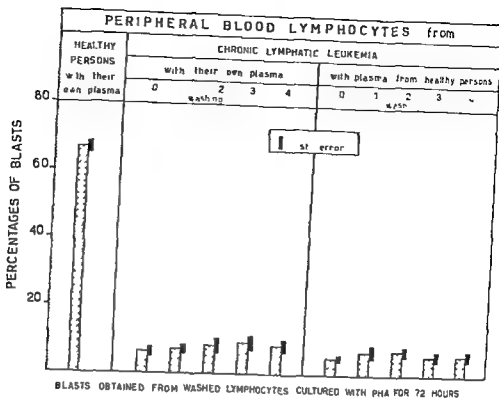


Fig 2

51, when the CLL lymphocytes were washed 1 2 3 and 4 times and then cultured with normal plasma and 7.0 ± 5.9 , 7.5 ± 5.5 , 5.7 ± 5.9 and 6.6 ± 4.8 when the same washed lymphocytes were cultured with their own plasma. In other words the number of blasts obtained in the experimental and in the control groups was of the same order of magnitude.

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The defective reaction of lymphocytes from CLL to PHA stimulus in tissue culture is a well documented phenomenon, whereas the causes why the lymphocytes of these patients give so low percentages of blasts in culture with PHA are not yet definitely established.

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Peripheral blood lymphocytes from CLL patients washed 1—4 times and then cultured in a medium added to plasma from healthy persons did not change their usual hyporesponsiveness to the PHA stimulation in tissue culture

Finally the CLL blood lymphocytes reacted normally to the PHA stimulation in culture after the spleen irradiation of the CLL patients

The above summarized results are discussed

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a medium containing plasma from normal individuals, gave always low percentages of blasts. This means that the performed washings have not changed the usual PHA-unresponsiveness of CLL lymphocytes, and thus that they should be PHA-non responsive because of their innate structure. At the same conclusion arrived Schreck (4), Robbins (14), and Oppeheim (12).

The incapacity of the serum from CLL patients to cause inhibition of the blastic development of lymphocytes from healthy persons suggests that the factor responsible for such an inhibition gets lost during the coagulation process, since it is present in the plasma and not in the serum.

Studies carried out by one of us (G Costa) have shown, that the inhibiting factor present in the CLL plasma is thermolabile (30' at 56° C), disappears during storage for 2 weeks in the refrigerator at 4° C and that it is destroyed by the X-ray irradiation "in vitro" (500 r). Moreover we have been able to show that irradiation of the spleen of CLL patients enables the lymphocytes from these patients to develop blasts after PHA stimulation in a percentage as high as that obtained in culture of lymphocytes from healthy persons (17, 18, 19). We have also observed that the plasma of these irradiated patients loses its capacity of inhibiting the PHA-responsiveness of lymphocytes from healthy persons (unpublished data).

In any case the results of these experiments, taken as a whole show that

1) the plasma from CLL patients contains a factor capable of inhibiting the blastic development of lymphocytes from healthy persons, when these lymphocytes are stimulated with PHA in tissue culture,

2) the defective response to PHA shown by CLL lymphocytes can be connected with the structure of these cells and may depend on the fact that these lymphocytes contain (produce?) within their structure the inhibiting plasmatic factor,

3) the factor responsible for such an inhibition gets lost during the coagulation process, since it is present in the plasma and not in the serum,

4) the above mentioned factor is thermolabile (30' at 56° C), it disappears after 2 weeks at 4° C, it is destroyed by the X-ray irradiation (500 r) directly delivered "in vitro", and finally it is removed "in vivo" by the spleen irradiation of the CLL patients.

Summary

The addition of plasma from chronic lymphocytic leukemia (CLL) to the PHA culture medium in which lymphocytes from healthy persons are cultured caused the inhibition of the blastic development of these cells. On the other hand, the serum from the same CLL patients had no effect in inhibiting the normal lymphocytes in the PHA culture system.

The same CLL plasma appeared to be no more inhibiting when kept at 56° C for 30' minutes and at 4° C for 2 weeks as well as after X-rays irradiation in vitro with 500 r.

Peripheral blood lymphocytes from CLL patients washed 1—4 times and then cultured in a medium added to plasma from healthy persons did not change their usual hyporesponsiveness to the PHA stimulation in tissue culture

Finally the CLL blood lymphocytes reacted normally to the PHA stimulation in culture after the spleen irradiation of the CLL patients

The above summarized results are discussed

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Myelofibrosis Associated with Tuberculous Lymphadenitis

BY SVEN MÅRTEN SAMULLSSON, ANDREAS HILLANDER, IVAR WERNER
and BJÖRN STENKVIST

Myelofibrosis is a relatively rare disease in this country and nowadays the same holds true also for tuberculosis. It was therefore unexpected to find tuberculous infection in four of the ten patients with myelofibrosis treated at this department during the last five years. The simultaneous occurrence of tuberculosis and myelofibrosis has been reported in several cases, the majority of which had generalized tuberculosis. This led in many authors to suggest that tuberculosis was the predisposing factor to the bone marrow changes. In our series cervical lymphadenopathy was the first obvious sign of active tuberculous infection. In one patient military tuberculosis developed later. The high incidence of tuberculosis in this series has prompted us to report the findings and to discuss the problems entailed in diagnosis and management.

Case reports

Case 1 A H house wife born 1901. One child who had pulmonary tuberculosis at the

age of 16. In 1914 the patient had joint pains during one month. She was in good health until 1931 when she had a new period of stiffness and pain in the large joints. The pains recurred in 1937 at which time a moderate anaemia (Hb 10 g%) was noted. In 1939 she was admitted to a hospital for observation. There were no joint deformities. Hb was 10 g%. White cells 1200—3000/mm³ with about 40% neutrophils and a platelet count between 140 000 and 200 000/mm³. The sedimentation rate was about 60 mm one hour. The sternal marrow showed toxic changes with some features resembling early megaloblastoid degeneration. No enlarged lymph glands were found. During the hospital stay she had a few attacks of fever which were treated with daily doses of 50—250 mg of cortisone and four blood transfusions. She was discharged after 3 weeks and was in good health for the following two years except for slight joint complaints. The blood picture however remained unchanged.

In July 1961 she began to feel weak, tired and hoarse. The temperature was about 38°C with some peaks up to 41°C. She perspired very much especially at night.

An enlarged supraclavicular lymph gland from the right side was removed. The histological examination showed a granulomatous tissue consisting of epithelioid cells growing in bands or more diffusely. Scattered small fibrinoid or possibly caseous necroses were

seen No acid fast bacilli or fungi were found Although the picture was compatible with *low dose* *sarcoidosis* and *tuberculosis* as more in favor of the former diagnosis X-ray of the lungs showed a moderate broadening of the mediastinum on the right side some small calcifications of the right hilar region and a small opacity in the left apical region

In September 1961 her condition deteriorated. Blood examination now showed Hb 62 g% red cells 20 mill/m³ reticulocytes 17-19% platelets 2000-3000/mm³ and white cells 3000-6000/mm³ with 40-50% mononuclears mostly lympho and monocytes some of them atypical a few erythroid mature cells containing one or two nucleoli and some nucleocytes and erythroblasts Sternal puncture yielded only blood and a few narrow cells Both erythropoietic and myeloid precursors showed maturation disturbances Tuberculin skin test was positive for 01 mg An enlarged cervical lymph gland was removed Examination showed abundant miliary tubercles with epithelioid cells some giant cells and widespread caseous necroses No acid fast bacilli were found The picture was compatible with tuberculous lymphadenitis No tubercle bacilli were found in sputum specimens and in cross-section of after guinea pig inoculation Treatment was started with streptomycin soon as diuretic amilorone and blood analysis showed her there was no improvement during the following two months She was transferred to the University Hospital in Lppsala in November 1961

On examination a few pea sized hard cervical lymph glands were noted The liver and spleen were not palpable The lung fields were clear and the right side broadening of the mediastinum had decreased The spine and the pelvis appeared normal on X-ray examination Hb as 89% platelets 20000-60000/mm³ and white cells 8000/mm³ About 80% of the leucocytes were mononuclear with the appearance of blast cells with nucleoli and a clear blue cytoplasm About 50 rhabdoid cells per 100 leucocytes are found Heptate punctures of the sternum and acetabula were too hypocellular to permit adequate judgment A surgical biopsy

from the iliac crest revealed pronounced myelofibrosis with a few islands of erythrocytes and myelopoiesis Some megakaryocytes were present Besides a number of large mononuclear cells of reticular type sometimes with monstrous nuclei were found No tubercles were observed The subsequent course was characterized by repeated febrile attacks increasing anaemia and thrombocytopenia with bleeding tendency No enlarged lymph glands were noted but the spleen became palpable in February 1962 Blood cultures were consistently negative Treatment with tuberculo-statals and bolus corticosteroids and elliptal testosterone was without effect The Sabin Feldman dye test for toxoplasmosis was positive in the titre of 1:20 and the complement binding test in 1:40 in the middle of February Two weeks later the titres were 1:120 and 1:20 respectively Repeated X-ray examination of the lungs still showed clear lung fields The tuberculin sensitivity had decreased but a positive reaction was still obtained with 3 Ag. Streptomycin and isoniazid were discontinued A new guinea pig inoculation with gastric contents as performed in the middle of February The clinical condition deteriorated further and the patient expired on March 3 1962 The last guinea pig inoculation showed no signs of tuberculosis

Autopsy revealed many tuberculosis affecting the lungs larynx liver spleen kidneys lymph nodes dura mater and bone marrow Guinea pig inoculation the specimens from the hilar glands gave positive reaction for tuberculosis The same picture as earlier of pronounced myelofibrosis was found As previously noted a small number of large reticular cells were observed Similar cells were also found scattered in the lymph nodes and the spleen

Case 3 GF lumberer born 1903 In 1930 he had rheumatic fever with pancarditis and bilateral pleural effusions After treatment with ACTH during one month he recovered completely The tuberculin skin test was positive for 01 mg

In Dec. 1961 he noticed swollen cervical glands on both sides His appetite was bad

Survey of cases with myelofibrosis and tuberculosis

Author(s) and year	Sex	Age years	Spleno megaly	Hepato megaly	Enlarged lymph glands	Leucocytes /mm ³	Comments	Platelets /mm ³	Duration of illness months		Tbc type	
									Myelo fibr	Tbc	Miliary	Non miliary organs involve ments
Donhauser, 1908	m	68	++	+	—	11550	no blasts	?	4	?		mesent gl (+ fibr pleurisy)
Dyke 1924	m	34	+	?	?	121000—2800	at first considered as myeloid leukaemia	?	6	3		+ (extends bone marrow invol)
Kraske & Nothnagel 1925	m	45	++	+	+	27000—4200	myeloblasts 9% promyelocytes 4% myelocytes 10% Myeloid leukaemia?	?	24	48?		+ (wide spread)
Wool et al 1933	m	III	++	—	—	22000	no blasts	?	?	few months		spleen
Hugonot & Solier 1935	m	64	++	++	+	11000	erythroblasts 1% myelocytes Polycy themic marrow features?	?	20	6?		bl spleen liver
Stone & Woodman 1938	f	43	++	++	?	94500	myeloblasts 1% Myelofibr devel 13 years after the diagn of polycyth	128000	>12	?		cerv and liver bl
Carpentier & Flory 1941	m	37	++	++	+	3100—86300	primitive cells 0—4% large platelets	++	40	?		wide spread
Herr & Irubling 1947	m	40	+	+	—	2000	1% myeloblasts	37000	< 6	6		lungs and mesent bl
Crail et al 1948	f	39	++	+	+	6000	erythroblasts 2% myelocytes	178000	18 (166?)	17		General
	m	50	++	++	+	3500—11000	5% myeloblasts normoblasts	251000	18 (28?)	?		liver spleen bone

	f	70	+	+	+	1700—11,000	60% myeloblasts (before death) Giant platelets and megakaryocytes		acute
Mikane & Ippolito 1918	f	49	+	+	+	3100—12800	no immature cells	4,00000	7 mediast gl + liver
Black & Jacobsen 1950	f	25	+	+	+	6000		?	2 acute miliary
Wells & Sommers 1950	f	61	+	+	—	7000	13% myelocytes	125000	18 120
Tsafirénuv et al 1951	m	62	+	+	?	7000	?	?	>30 ?
Festiparmuk cu et al 1957	f	63	+	+	+	13000—2,000	1% myeloblasts 4% myelocytes 7% blasts 8% myelocytes	?	12 (alive) 7 (alive)
André et al 1961	m	64	+	+	+	21000	5% myelocytes	550000	48 (alive)
	f	62	+	+	+	21400	1% promy 1% myelocytes Myelof devel 3 yrs after diag of polycyth	1000000	144 (alive)
	m	65	+	+	+	17600	probabls also polycythemia	100000	14 (alive)
Anderson et al 1961	?	?	+	+	+	213000	exposure to ionizing radial of atomic bomb Terminal ilic	1,00000	50 ?
	?	?	+	+	+	187200		141000	54 ?
	?	?	+	+	—	15800	exposure to ionizing radiation of atomic bomb	674000	9 ?

lungs

right lung

spleen

cerv gl

lungs +
cerv gl

+

+

+

and he had lost weight. In February 1962 he was admitted to the medical department.

Physical examination disclosed several firm lymph nodes varying in size from a pea to a plum on both sides of the neck. The liver edge was felt about 3 cm below the right costal margin and the spleen just below the left costal margin. The temperature was normal.

On X-ray the lung fields were clear. The lumbar spine and the pelvis showed a generally increased density.

The tuberculin test (0.1 mg) was positive. No acid fast organisms were found in a sputum specimen and gastric contents produced no tuberculosis in the guinea pig. Hb was 9.3 g%, red cells 3.5 mill/mm³, reticulocytes 2.8%, platelets 440,000/mm³ and white cells 7,200/mm³. A differential count showed 5% promyelocytes, 5.5% myelocytes, 8% metamyelocytes, 19% band forms, 47% segmented neutrophils, no eosinophils, 1% basophils, 12.5% lymphocytes and 2% monocytes. A few nucleated red cells were also found. Serum iron was 32 µg%, transferrin 173 µg% and haptoglobin 418 mg% (normal value 30—190 mg%). The alkaline and acid phosphatase activity of the serum was normal. Vitamin B₁₂ in serum was normal.

No marrow was obtained on repeated aspirations from the sternum and iliac crest. A surgical biopsy from the iliac crest showed myelofibrosis with abundant connective tissue with very few marrow cells. Splenic puncture showed numerous erythroblasts as well as some myeloid precursors.

Histological examination of a cervical lymph node showed many tuberculous granulomas with central necrosis and epithelioid cells and a few giant cells of Langhans type in the periphery.

A diagnosis of cervical gland tuberculosis and myelofibrosis was made. The patient was treated with streptomycin for two months followed by isoniazid for one year. During this therapy the cervical glands diminished in size. In May 1962 the patient was given a series of six injections of 50 mg nortestosterone each resulting in some subjective improvement. One month later he was working

full time and Hb was 10.4 g%. On repeated controls during the following two years there was no significant change in his condition.

In July 1964 he had an upper respiratory infection following which he felt increasingly tired, weak and dyspneic. On readmission in August numerous grape sized hard subcutaneous nodules were palpable all over the body. The edge of the liver and spleen extended below the umbilical level. Hb was 9.7 g%, white cells 6,800/mm³ with about the same distribution as in 1962. Platelets were 80,000/mm³. Sternal marrow was now obtained. The smear was hypocellular containing mostly erythroblasts and large reticular cells with pronounced polymorphism and many nucleoli. The impression of a reticulum cell sarcoma was confirmed by the histological examination of an excised skin tumour. Treatment with cytostatics and steroids was ineffective and the patient expired on Sept. 28. Autopsy revealed wide spread reticulum cell sarcomatosis, osteomyelofibrosis but no signs of active tuberculosis as had been found previously.

Case 3. A.G. carpenter born 1889. Since 1936 he had suffered from repeated phlebothromboses in the legs. In 1960 splenomegaly was discovered. Hb was 12.5 g%, white cells 22,000/mm³. A few myeloid precursors and nucleated red cells were seen in the blood smear. The platelet count was normal. In 1961 a right side renal tumour and ventricular polyposis were diagnosed. In August 1962 an enlarged supraclavicular lymph node was observed on the right side and X-ray of the lungs showed enlargement of the hilar region. Microscopically the gland contained numerous caseous necroses surrounded by epithelioid cells and giant cells of Langhans type. No tubercle bacilli were found in sputum specimens or after guinea pig inoculations with gastric contents. The patient was treated with streptomycin, PAS and isoniazid. The blood picture had not changed significantly since 1960.

In March 1963 the patient was admitted to the University Hospital. On both sides of the neck groups of pea sized lymph nodes were

palpable. The liver was felt about 7 cm below the costal margin. The spleen reached to the iliac crest. Below the liver a grapefruit sized tumour was felt. Hb was 14.2 g% red cells 5.1 mill/mm³ haematocrit 32-38 % reticulocytes 12-18 % and white cells 20 000-32 000/mm³. Differential count myeloblasts 6% promyelocytes 3.5% myelocytes 3.5%, metamyelocytes 20% band forms 8.5% segmented neutrophils 61% eosinophils 3.5% basophils 40% lymphocytes 60% and monocytes 20%. A few erythroblasts were found. Platelets were 59 000-108,000/mm³. Sedimentation rate 1 mm/one hour. Sternal marrow aspiration was difficult. A few fragments were obtained which made histological examination possible. The marrow was hyperplastic. The myelopoiesis — and to a lesser degree the erythropoiesis — showed great activity. Many megacaryocytes were seen. Streaks of collagen fibres, fibroblasts and many vessels were seen indicating early marrow fibrosis. Splenic puncture showed myeloid metaplasia. Total haemoglobin and blood volume were moderately increased to 911 g (12.3 g/kg body weight) and 887 litres (19.5 ml/kg body weight) respectively. The staining of leucocytes for alkaline phosphatase according to Kaplow showed strongly positive granulocytes. The heart volume was 830 ml/m² body surface. The hilar region was now of ordinary appearance. The tuberculin skin test was positive for 1 mg. X-ray examination of the stomach showed wide spread polypoid. Renal aortography showed the right renal tumour to be a large cyst. The treatment with isoniazid was continued and the patient was discharged in a rather good condition.

The patient was later admitted to a hospital for chronic diseases. In September 1964 he died of congestive heart failure. Autopsy was performed but adequate histological evaluation was impossible because of advanced postmortal autolysis.

Case 4 EF farmer born 1899. A sister had died of pulmonary tuberculosis about 1940. Apart from some periods of manic depressive psychosis the patient was healthy until 1950 when a pronounced splenomegaly

was found during a period of treatment in the psychiatric clinic. Subsequent investigation at the medical department revealed a spleen extending down to the iliac crest. The liver was palpable about 7 cm below the costal margin. No lymph node enlargement was found. X-ray films of the lungs showed small apical calcifications. Within the whole spine pelvis humeral and femoral bones wide spread areas of osteosclerosis were seen. Hb was 12.8 g% white cells 6 900/mm³ with a few myeloid precursors and erythroblasts. Platelets were 150 000/mm³. Surgical biopsy from the iliac crest showed typical osteomyeloid metaplasia with a hypoplastic marrow. Splenic puncture showed myeloid metaplasia.

After leaving the hospital the patient felt well and did not return for control examination until 1959. After an upper respiratory infection he felt tired, lost appetite and complained of perspiration especially at night. The spleen extended down to the pubic region. Hb was 10 g% red cells 3.4 mill/mm³ white cells 2 400-4 400/mm³ and platelets 130 000/mm³. A few myelocytes, metamyelocytes and erythroblasts were found in the peripheral blood. Without any treatment he improved. In 1960 cholecystectomy was performed because of cholelithiasis.

In the spring of 1962 he became increasingly tired, dyspnoeic and lost weight. There was no fever. In August 1962 he was readmitted to the medical department. He was now emaciated. A few date sized lymph nodes were now palpable on the left side of the neck. The liver edge was felt about 9 cm below the costal margin and the spleen filled up the whole abdomen on the left side. Hb was 6.1 g% red cells 2.6 mill/mm³ white cells 4 200/mm³ and platelets 49 000-94 000/mm³. The differential count was unchanged. The tuberculin skin test was positive for 1 mg. X-ray films of the lungs were normal. Treatment with steroids, methyl testosterone and nortestosterone had no effect. The patient expired in January 1963.

Autopsy showed pronounced osteomyeloid metaplasia and haemopoiesis in liver and spleen. On the left side of the neck 3-6 cherry sized firm lymph nodes were found. Microscopic

pically they showed tuberculoid caseous necroses surrounded by granulation tissue. No other sign of tuberculous infection was noted.

Discussion

All four cases showed a histological picture typical for tuberculosis with tubercles with Langhans cells and caseous necroses. For some reason no acid fast bacilli were found and unfortunately cultivation or guinea-pig inoculation were not made on autopsy material in three cases. The typical histological findings, however, in cases 2, 3 and 4 and the positive result with tuberculostatic treatment in cases 2 and 3 strongly support the diagnosis of tuberculosis.

The diagnosis of myelofibrosis seems unequivocal in cases 1, 2 and 4. The histological picture of biopsy specimens and autopsy findings were typical. The diagnosis in case 3 requires some consideration. Obviously his final disease started with the clinical picture of polycythaemia vera. At the time of discovery of the tuberculosis the picture was still suggestive of a mild polycythaemia. Biopsy specimens of his bone marrow showed marked hyperplasia but at the same time there were streaks of collagen fibres, many fibroblasts and blood vessels, strongly suggesting incipient myelofibrosis.

A striking feature in our series is the localization of the tuberculous infection to the lymph glands, predominantly in the neck region. Only about 10% of all cases of active tuberculosis in our area have this localization (10). The explanation might be that the

resistance of the lymphoid tissue to tuberculous infection is impaired by the myelofibrotic process.

Another possibility is that owing to the effective and thorough public health control by means of regular mass X ray examinations of the lungs in Sweden pulmonary tuberculosis rarely escapes detection and treatment at a very early stage. On the other hand tuberculous infection of some lymph nodes might possibly remain undiscovered for many years. The occurrence of a debilitating disease, such as myelofibrosis, may activate a latent tuberculous infection. A high frequency of tuberculous lymphadenitis would then be expected in our country also in other debilitating conditions. As far as we know this is not generally the case.

Morrow and Anderson performed an extensive study on active tuberculosis in malignant lymphoma and myelofibrosis in an autopsy material from Hiroshima 1949—1962 (14). No instance of associated active tuberculosis was found in 37 cases of malignant lymphoma despite the fact that the majority of these cases were associated with debilitation and had had a protracted clinical course. In contrast to this disseminated tuberculosis was found in association with chronic myelogenous leukaemia (43 cases) and myelofibrosis (12 cases) where the prevalences were 2.5 and 4.5 times respectively that found in the remainder of the autopsy population. Thus it seems improbable that the debilitating state per se is the essential mechanism

behind the occurrence of tuberculosis in myelofibrotic states

The simultaneous occurrence of myelofibrosis and tuberculosis has been reported in several papers. The relevant findings of the cases described are listed in the table

Some authors have suggested that the myelofibrotic process might be a reaction to the tuberculous infection. It is evident that the patients represent a heterogeneous group. In some cases (1, 2, 4, 5, 11, 13, 18) the myelofibrosis preceded tuberculosis by one or more years. The same holds true for case 4 in our series. Most of the patients, however, developed symptoms of both diseases either simultaneously or within a few months. Cases 1 and 2 of our series probably belong to this group. The possibility cannot be excluded, however, that the moderate anaemia and leukopenia found in case 1 and known two years before the appearance of the tuberculosis might have been the first sign of myelofibrosis. As shown in the table, there is a great variation in the number of leucocytes, platelets and immature cells in the peripheral blood. Thus it may be suggested that the association of myelofibrosis and tuberculosis does not represent an aetiological entity.

The simultaneous occurrence of the two diseases is, however, far too common to be merely coincidental. This is especially apparent in a community like ours, where both conditions are rare. Among the explanations offered — aetiological, statistical and others

— the most probable seems to be that the myelofibrotic disease may diminish the resistance to the tuberculous infection.

From the practical point of view the coexistence of the two diseases offers certain difficulties in the diagnosis and managements of the patients.

The symptoms of tuberculous infection may easily be overlooked. This is especially true when the pulmonary findings are unobtrusive or missing altogether. Symptoms such as fever and chills, sensitivity to infections, tiredness, malaise, weight loss, moderate anaemia and granulocytopenia, elevated sedimentation rate and the appearance of lymph node and spleen enlargement may all be explained by the myelofibrotic process. To judge from our series it is apparently difficult to obtain a positive bacteriological diagnosis. Microscopic examination of sputum or guinea pig inoculations with gastric contents were constantly negative. It must be stressed that histological examination of biopsy specimens should always be performed when unexpected lymph node enlargement appears in myelofibrosis. However, our experience from the first patient (A.H.) demonstrates that histological diagnosis may also be very difficult.

There is no specific treatment of myelofibrosis. Supportive treatment with androgens, blood transfusions and antibiotics in cases of infection are the principal measures. Radiotherapy and busulphan may be used in selected cases. During the last decade cor

lisonc or cortisone derivatives have been used more frequently. This is especially dangerous in the presence of latent tuberculosis. The milirary spread of the tuberculous infection in our case I was most probably enhanced by the discontinuation of the tuberculostatic medication during the corticosteroid treatment. In our opinion even suspicion of tuberculosis in myelofibrosis is a good reason for the institution of long and intensive anti-tuberculous therapy. This is especially important when corticosteroids are used.

Summary

In a series of ten cases of myelofibrosis no less than four had active tuberculosis. Cervical lymphadenopathy was the first obvious sign of the infection. Earlier reports of 24 cases with myelofibrosis and tuberculosis are reviewed. The possible relationship between the diseases is discussed. Both diagnosis and treatment of tuberculosis may be difficult in myelofibrosis. Histological examination should always be performed when lymph node enlargement appears. Adequate anti-tuberculous treatment should be instituted on the slightest suspicion of tuberculous infection in myelofibrosis.

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Primary Polycythaemia Associated with Multiple Myeloma

By SIVTEN FRANZEN, BO JOHANSSON, MAI KAIGAS

During the past fifteen years 293 patients suffering from polycythaemia vera and 132 patients suffering from multiple myeloma have been admitted to Radiumhemmet. Among these patients three cases with primary polycythaemia associated with multiple myeloma have been diagnosed. This association is very interesting in view of the discussion of a possible relationship between chronic myeloproliferative disorders and plasma cell tumours (1, 2, 4, 5, 7, 8). Some reports of patients with multiple myeloma and concurrent polycythaemia vera have been published, but only in a few cases have the two diagnoses been well established. We therefore, find it of value to report the following three cases.

Case 1 (Fig 1) a 63 year old female who was admitted to Radiumhemmet in May 1958 with a 5 year history of weakness, shortness of breath, warm and sweaty skin and several episodes of spontaneous epistaxis yearly. In 1957 her local doctor observed an elevated red blood cell count and an enlarged spleen. Her past history was noncontributory.

Physical examination revealed a round bodied female with a ruddy complexion. The spleen was palpable three fingerbreadths below the left costal margin.

Laboratory data. The hematocrit value was 51 %, red blood cell count 5 200 000 per cum mm, white cell count 11 400 per cum mm, platelet count 310 000 per cum mm. The sedimentation rate was 6 mm per hour. The blood volume was found to be 4.9 liters compared with a calculated normal value of 3.6 liters. Total serum proteins were 8.2 gm per 100 ml with albumin 4.0 gm per 100 ml and globulin 2.7 gm per 100 ml. Serum electrophoresis showed a narrow band of abnormal protein in the gamma 1 area. Urine was negative for Bence Jones protein.

Bone marrow scintigram was performed by injecting colloidal ^{199}Au intravenously (6). Pronounced uptake was found in both the trunk and the long bones (Fig 2).

X rays of the bones revealed a slight osteoporosis and a slight compression of two vertebrae. No local destructive lesions were found.

Bone marrow biopsy (sections and smears Fig 3). Only a few fat cells were observed in the marrow fragments. The trabeculae were crowded with granulocytopenia and erythroblasts. Huge bizarre megakaryocytes were common and sometimes gathered into groups. Some solid proliferations of plasma cells with malignant nuclei ("myeloma cells") were also observed.

Course. The patient was treated with 11 mC radioactive phosphorus immediately after admission in 1958. She showed a definite symptomatic improvement. All series of blood cell counts decreased in number and the spleen in size. In October 1963 she developed

POLYCYTHAEMIA VERA + MULTIPLE MYELOMA. H.F. FEMALE 63 YEARS

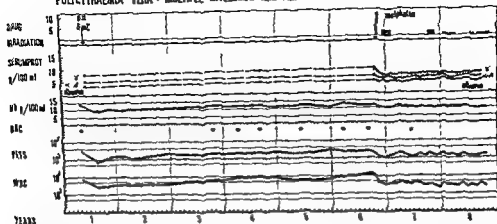


Fig 1 Case 1 The blood cell counts in this diagram are plotted in a semilog scale. The dotted areas delineate the normal range of blood counts for the respective components. The very slow increase of abnormal globulin during 1958-63 is well illustrated. The dark field means total gamma protein values. The responses to radioactive phosphorus and melphalan is very striking. Note that melphalan is administered in such doses that the blood cell counts follow the lower limit of the normal range of values.

a recurring pain in the lower dorsal spine. The roentgenograms at that time showed a pronounced compression of two vertebrae. She was treated with local radiotherapy and the pains disappeared. Treatment with melphalan was also started. The patient responded to this treatment with a rather sudden drop in the blood values. The abnormal globulin also decreased very rapidly. Thereafter the patient received maintenance doses of melphalan. Today the patient is symptom free and is able to do her house work. All blood cell counts are within the normal range. Her serum electrophoresis still shows abnormal globulin but the serum globulin value has decreased to 1.7 gm per 100 ml. The roentgenograms show no distinct signs of destructive lesions. In the bone marrow no signs of primary polycythemia can be found but there still exist solid proliferations of malignant plasma cells at about the same extent as before treatment. The bone marrow scintigram two years after the start of treatment reveals a less visible uptake of colloidal gold in the marrow of the long bones (Fig. 2).

Summary The patient had a marrow picture consistent with the diagnosis of primary polycythemia and multiple myeloma. The symptoms resulted from the polycythemia which was treated with radioactive phosphorus with good results. The myelomatosis was symptom free for more than 5 years. When the patient developed symptoms from this disease in 1963 she was treated with a combination of local radiotherapy and melphalan. This treatment has to date stopped the progression of the myelomatosis as well as of the primary polycythemia.

Case 2 (Fig. 4) a 80 year old male who was admitted to Radboudhemmel because of multiple myeloma in November 1964. The patient's history revealed a thrombosis in a leg a few years prior to admission. The red blood cell count was normal as late as 1962 at which time he sought medical care for

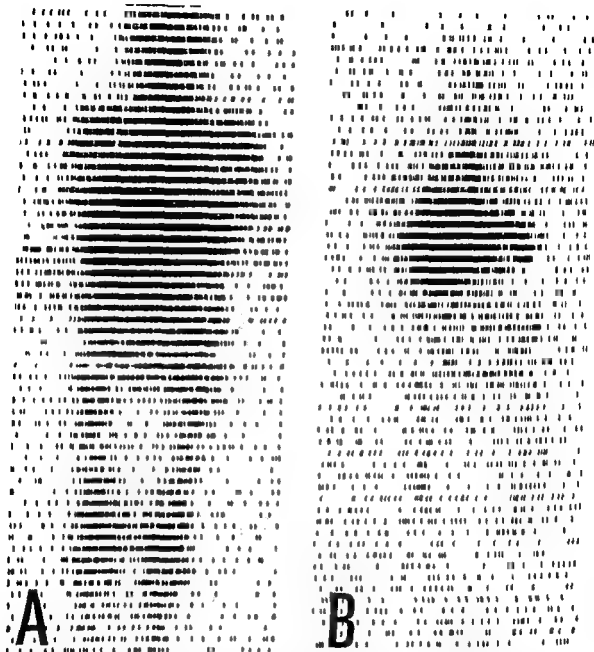


Fig 2 Case 1 Bone marrow scintigrams A Before treatment with melphalan Pronounced uptake of Au¹⁹⁸ in the marrow of the trunk and long bones B Two years after the start of cytostatic treatment The uptake of Au¹⁹⁸ is less pronounced in the marrow of long bones Because the used technique is not exactly the same in the two scintigrams a direct quantitative comparison is not possible

dizziness He improved on symptomatic treatment After direct questioning he revealed itching after warm baths In September he fell acutely ill of pneumonia and was hospitalized After treatment with antibiotics he

improved rapidly Because of his plethoric appearance and a high red blood cell count a marrow biopsy was performed

Physical examination on admission revealed a highly plethoric patient in good general

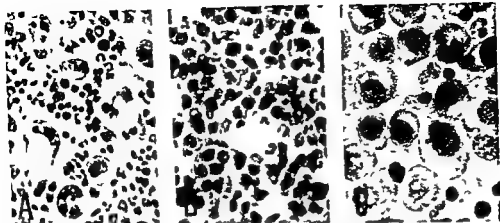


Fig 3 Case 1 Sternal marrow. A: Histologic section of fat free particles with hyperplasia involving all the marrow elements. Note the bizarre megacaryocytes. This picture characterizes primary polycythaemia (ref 3). B: Other marrow particles consist of solid proliferations of myeloma cells. (B: histologic section C: smear)

POLYCYTHAEMIA VERA + MULTIPLE MYELOMA E.L. MALE 80 YEARS

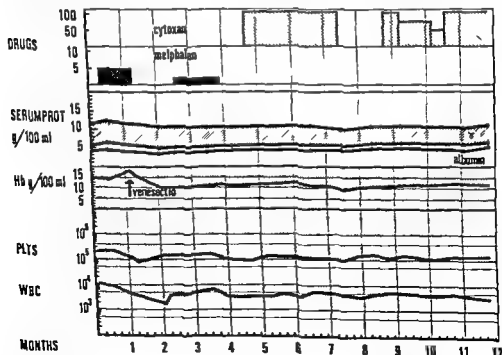


Fig 4 Case 2 The decrease of abnormal globulin is very gradual. Note the different effects of melphalan and cytoxan on the platelet counts. The effect on the two diseases of cytostatic drugs is well shown.

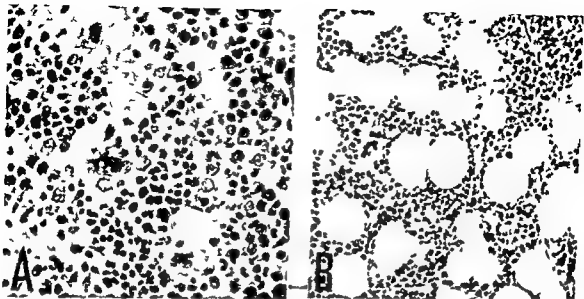


Fig 5 Case 2 A Histologic section of fat free marrow fragments before treatment The lower left part contains all blood cell precursors Note the bizarre megacaryocyte To the right proliferation of malignant plasma cells B Sternal marrow seven months after the start of treatment The fragments contain normal amount of fat cells No signs of primary polycythemia but still solid proliferations of "myeloma cells" up to the right

condition The spleen and liver were not enlarged

Laboratory data The hematocrit value was 50% red blood cell count 5 900 000 per cu mm white blood cell count 10 600 per cu mm platelet count 241 600 per cu mm sedimentation rate 0 mm per hour total serum proteins 122 gm per 100 ml with albumin 37 gm per 100 ml and gammaglobulin 71 mg per 100 ml Serum electrophoresis revealed a heavy spot of abnormal globulin in the gamma 1 area There was no Bence Jones protein in the urine The x ray studies of the bones demonstrated some suspicious but no certain destructive lesions of the ribs

Bone marrow biopsy (Fig 5) Fat cells were rare Pronounced hyperplasia of the erythropoiesis and granulocytopoiesis Megacaryocytes of hyperplastic types were numerous Several solid proliferations of malignant plasma cells were observed

Course Treatment with melphalan was started in a daily dose of 5 mg After an initial fall of all blood values there was a sudden rise of the hemoglobin values The white cell count and platelet count continued

to decrease At this time the patient felt very ill with severe vertigo as the chief complaint We found it necessary to perform two phlebotomies of 400 ml each The cytostatic treatment was discontinued for a time and later reinstituted with doses of only 2 mg daily This lower dose also produced a suppression of the platelet count to subnormal value without any significant decrease of the abnormal protein Therefore we changed to cytoxan which was given from April 1965 in doses of 100 mg daily On this course of treatment all blood values returned to and remained within normal limits The abnormal globulin seems to decrease very gradually The sedimentation rate that initially was 0 mm per hour increased at the same time the hematocrit value was normalized and has thereafter been 20 to 30 mm per hour The patient is today symptomfree

Summary The patient had a marrow picture consistent with a diagnosis of primary polycythemia and multiple myeloma Because of the very

POLYCYTHAEMIA VERA KS FEMALE 51 YEARS

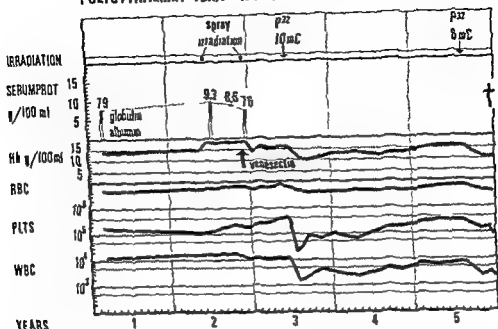


Fig 6 Case 3 This case illustrates the normal response to radioactive phosphorous in a patient with primary polycythemia. Only three estimations of serum protein were performed.

high value of abnormal serum protein melphalan treatment was started. The effect of this drug on the platelets however was too severe to allow a dose high enough to stop the progression of the myelomatosis. Cytosin the rapy was therefore started. With this drug, it has been possible to obtain a decrease in the abnormal protein. Blood values have returned to normal limits. The cytostatic therapy has to date made the patient free from signs of primary polycythemia and also seems to have started a regression of the myelomatosis.

Case 3 Fig 5 53 year old female who was admitted to Radiumhemmet in July 1951 because of primary polycythemia. The pa-

tient's past history revealed that she had enjoyed good health until 1951 at which time she complained of weakness that made her unable to work after October 1952.

On admission to her local hospital in June 1953 physical examination revealed a red dish cyanosis of the face. She complained of itching in the eyes and her conjunctivae were intensely injected. The liver and spleen were enlarged.

Laboratory data. The hematocrit value was 80%, red blood cell count 5900000 per cu mm, white blood cell count 14100 per cu mm, platelet count 118000 per cu mm, sedimentation rate 8 mm per hour, total serum proteins 9.3 gm per 100 ml, with albumin 4.6 gm per 100 ml, and globulin 4.7 gm per 100 ml. A probable diagnosis of polycythemia vera was made.

Course. In June and November 1953 the patient was treated with total body irradiation (spray irradiation). She did not improve.

and was admitted to Radiumhemmet in June 1954. On admission the spleen was enlarged. The hematocrit value was 39%, white blood cell count 13 400, platelet count 419 000. Bone marrow biopsy revealed no fat cells and a maximal hyperplasia of erythropoiesis and granulocytopoiesis. The enormous number of large megacaryocytes was very striking. The number of plasma cells was increased but no solid proliferations were found. In June 1954 the patient was treated with 10 mC radioactive phosphorus. She improved very rapidly. Because of signs of progression in October 1956 she was treated with 6 mC radioactive phosphorus with the same good results. In December 1956 the patient complained of pains in the spine radiating down the legs to the toes. She related that she had felt similar pains now and then since 1954. The symptoms showed a very progressive course and she developed weakness, paresis and numbness of the lower limbs. In the neurological department a cisternal puncture was performed to localize the lesion by a myelogram. Because of a bleeding caused by the examination the patient died. At necropsy the examiner beside the mentioned bleeding found an extradural expanding mass in the lower thoracic area. The tumour infiltrated the spinal nerves and compressed the spinal cord. Similar masses were observed in many vertebrae and in the right femoral bone. Other bones were not examined. The histological examination revealed the tumour to be a plasmacytoma.

Summary The patient had symptoms, blood values and a bone marrow picture consistent with the diagnosis of primary polycythaemia. After treatment with radioactive phosphorus she improved. She suddenly developed symptoms of compression of the spinal cord about two years after the initial diagnosis. At necropsy several plasma cell tumours were found in the bones. All earlier marrow biopsies have been reexamined. No definitive

signs of multiple myeloma have been found, not even in biopsies taken 6 months prior to death. The findings of high globulin values in 1954 and the patient's own history of her pains make it most probable that the malignant transformation of the plasma cells had started before this time.

Discussion

Patients suffering from two or more malignant tumours derived from different cell lines are of interest from many points of view. Especially different tumours belonging to the same organic system in one host will arise questions about etiological factors in malignant tumours. Besides the statistical possibility of coincidence, there may be other explanations to such an incidence. This may be due to a special constituent of the host making him more susceptible to malignant transformations. The possibility of a common unknown agent starting the malignant transformation must also be taken into consideration. Among clinicians the possibility that one tumour is the end result of the treatment of the other tumour has been widely discussed. Regarding the carcinogenic effects of radioactivity and alkylating agents such a discussion is very natural.

In the first two patients discussed here the coincidence of primary polycythaemia and multiple myeloma was diagnosed before any form of antitumour treatment was started. This is well illustrated by the marrow biopsies on admission. In the third case it

can not be proved that two diseases occurred concurrently but a retrospective study of the case history makes it probable that the myelomatosis had developed before the start of anti tumour treatment

We want to underscore the long survival time of case 1. A long survival time is generally accepted as consistent with the diagnosis of primary polycythaemia. The general opinion in regards to multiple myeloma is that this disease has a very bad prognosis although long survival times have been reported. In case 1 the myelomatosis has shown a very slow progressive course and was symptom free up to five years after the diagnosis. In our opinion the prognosis of multiple myeloma today is much better than earlier supposed. This is probably partly due to the earlier diagnosis and to the new methods of treatment.

Regarding the response to treatment our experience from the three cases shows the well known fact that radioactive phosphorus is very effective in the treatment of primary polycythaemia. From these cases it is impossible to draw any conclusions about the effect of radioactive phosphorus on multiple myeloma.

The alkylating agents melphalan and cytoxan seem to have effected the course of primary polycythaemia as well as that of myelomatosis. Of the two diseases primary polycythaemia has been most effectively improved. Although the effect on the myeloma

tosis is much less pronounced the treatment seems to have been of value to the patients.

Summary

Three cases with primary polycythaemia concomitant with multiple myeloma are presented. The possible relationship between the two diseases and the results of given treatments are shortly discussed.

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Occult Nontropical Sprue and Associated Atrophic Gastritis Simulating Addisonian Pernicious Anemia, With Special Reference to Immunologic Diagnostic Studies

BY PAUL BROWN, M D, KIRK D WUEPPER, M D, and H H GUDENBERG, M D

Introduction

The differential diagnosis of Addisonian pernicious anemia from other megaloblastic anemias has been facilitated by the use of radioactive vitamin B₁₂ uptake tests, serum vitamin B₁₂ determinations, and the recently introduced immunologic methods for detecting antibody to gastric parietal cells and intrinsic factor and for quantitating intrinsic factor production by the stomach. The incidence of antibodies to either parietal cells or intrinsic factor, or both has approached 90% in patients with uncomplicated Addisonian pernicious anemia (1-3), and 100% of such patients produce little or no intrinsic factor as measured by immunoassay (4).

The following patient with megaloblastic anemia due to an unusual

combination of atrophic gastritis and nontropical sprue illustrates the usefulness of these immunologic diagnostic methods and also raises a question of the possible interrelationship of the two diseases.

Case report

C J (UC # 3a1780) a 78 year old retired carpenter was born in Denmark and has lived in the United States since the age of 20. As a life long bachelor living alone in small apartments his diet was not above reproach but was not grossly deficient in sources of vitamin B₁₂ or folic acid. He has never taken alcohol, has had no abdominal operations and had not suffered a single serious illness in his entire life. In 1963 he was hospitalized for a cataract removal his hematocrit was 32% and a blood smear showed considerable anisopoikilocytosis and many macrocytes but the significance of these findings was not appreciated. Two months before admission for the present illness in 1965 he noted slowly progressive swelling of the legs and shortness of breath and one month before admission he had mild diarrhea for one to two weeks that subsided spontaneously. He walked into the hospital

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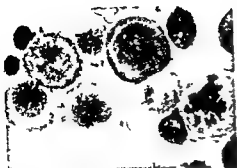


Fig 1 Bone marrow specimen from patient, showing characteristic megaloblastic erythrocytes and precursors. Wright-Giemsa stain. Original magnification $\times 800$.



Fig 2 Gastric biopsy specimen showing parietal atrophy and lymphocytic infiltration. Hematoxylin and eosin stain. Original magnification $\times 40$.

He was an elderly taciturn and mentally slow man with a pallid lemon-colored skin. Further examination revealed anasarca, bilateral pleural effusions, cardiomegaly, absent vibratory and diminished positional touch and pain sensation in both legs.

A blood smear showed extreme variation in size and shape of the red cells with numerous nucleocytes and nucleated red cells, target cells, hypersegmented neutrophils, and Howell-Jolly bodies. White cells and platelets were diminished and an occasional hypersegmented polymorphonuclear cell was noted. In the last bone marrow was frankly megaloblastic. Ferritin with almost no stainable iron. The white cell count was 400, platelet count 48,000, red cell count 1.09×10^{12} per liter, hemoglobin 3.7 gm/dl, and reticulocyte count was 0.6%.

Other studies of the patient on admission to the hospital were as follows: hemoglobin 10.0, hematocrit 30%, normal sedimentation rate 10-15 mm/hr, serum iron 150 $\mu\text{g}/100 \text{ ml}$, transferrin saturation 20%, serum ferritin 210 μg , serum LDH 3100 units, serum creatinine 1.2 mg/dl, test and retest urea nitrogen 10.4 and 10.0 mg/dl.

The urinary excretion of radioactive technetium-99m was 3% of normal, greater than 10%. Repeat Schilling tests with added intrinsic factor produced no effect, and no other pa-

tients with pernicious anemia resulted on two occasions in urinary excretion of 1% of the radioactive dose each time. Renal function tests were normal. Repeat bone marrow showed marked erythrocytic hyperplasia with complete disappearance of megaloblastic cells and a small amount of stainable iron. The stomach contained no free acid either before or after stimulation by a standard 50 mg dose of histalog. Gastrointestinal x-rays revealed only a slow gastric emptying time and a dilated small bowel. Stools were negative for ova and parasites. 10% of the ingested fat was excreted during a 3-day stool collection, normal less than 2%. A d-xylose uptake test showed 9% urinary excretion in 5 hours, normal more than 20%.

Antibody to gastric parietal cells was determined in our laboratory according to the method of Taylor and associates (3). Antibody to intrinsic factor and intrinsic factor estimation in gastric juice were determined by Dr. Keith Taylor using a micro modification of the method of Ardeman and Chanarin (6). No serum antibodies to either parietal cells or intrinsic factor could be detected, but intrinsic factor was demonstrable in a one-fifth collection of gastric juice following histalog stimulation. Biopsy of the stomach confirmed a mild atrophic gastritis, with moderate lymphocytic infiltration and approx-



Fig 3 Biopsy specimen of jejunum showing subtotal villous atrophy and lymphocytic infiltration. Hematoxylin and eosin stain. Original magnification $\times 50$.

imately 30% remaining parietal cells (Figure 2) and biopsy of the jejunum revealed a subtotal villous atrophy (Figure 3).

Idendum. After 3 months on a gluten free diet the patient was restudied. The serum carotene content had increased from 0.004 to 0.06 mgm%, the urinary excretion of D-xylose from 9 to 13% and the fat content of a 3 day stool specimen had decreased from 10 to 7%. The most striking improvement however had occurred in the absorption of vitamin B₁₂. A Schilling test (without added intrinsic factor) now showed urinary excretion of 16% of the radioactive dose in contrast to the previous value of 3%. A gastric biopsy now showed increased mucosal thickness, more prominent parietal cells and less inflammation than the previous biopsy. Thus improvement in both gastric morphology and function and in intestinal absorption have been clearly documented.

Discussion

Initially, many reasons existed for thinking that Addisonian pernicious anemia was the sole cause of this patient's illness. The abnormally low urinary excretion of vitamin B₁₂ appeared to support this initial diagnosis as did the demonstration of histamine fast achlorhydria.

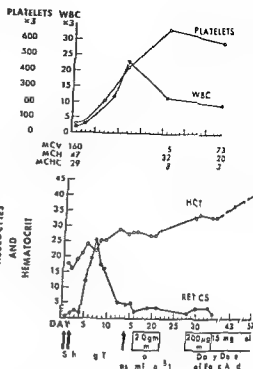


Fig 4 Patient C J Hematological course and pertinent therapy.

At this point however, three different pieces of evidence made the diagnosis of uncomplicated pernicious anemia untenable. First the patient's serum contained no antibodies to either gastric parietal cells or to intrinsic factor, second, a Schilling test with added intrinsic factor produced no increase in vitamin B₁₂ excretion and, third serum levels of both vitamin B₁₂ and folates were low. Attention was therefore directed to the possible existence of an unsuspected intestinal disorder and subsequent studies did indeed demonstrate malabsorption as associated with biopsy proven nontropical sprue.

Meanwhile the patient responded to the Schilling test with a dramatic reticulocytosis, gradual hematocrit rise and total elimination of megaloblastic

cells in the bone marrow. Subsequently physiological doses of parenteral folic acid induced a second small reticulocyte response which may have been dampened however, by the earlier large doses of vitamin B₁₂. Could the patients now well established combined vitamin B₁₂ and folic acid deficiency have developed solely on the basis of his small bowel lesion or was gastric disease also playing a part? The finding of achlorhydria had suggested that the stomach was not normal and a biopsy revealed the existence of mild atrophic gastritis but the contention that this condition contributed to the vitamin B₁₂ deficiency could only be proved by showing the absence of intrinsic factor in the patient's gastric juice and immuno assay documented its presence.

Immunological phenomena in pernicious anemia were at first considered to result from oral treatment with heterologous intrinsic factor (7). Taylor and associates subsequently noted a serum factor capable of combining with and inactivating intrinsic factor in untreated cases of Addisonian pernicious anemia (5). A by product of these investigations was the discovery of serum antibodies specific for the gastric parietal cell detectable both by complement fixation and immunofluorescence (8). Antibodies to intrinsic factor are present in approximately 50% of adults with pernicious anemia (1, 2) and fluorescent antibodies to parietal cells are found in about 90% of such patients (2, 3, 9). In our laboratory parietal cell fluorescent antibody has been demonstrated in 93%

of patients with uncomplicated Addisonian pernicious anemia.

Antibodies to parietal cells also occur in a small proportion of apparently healthy people in larger proportions of patients with diabetes mellitus and thyroid disease and in about 60% of patients with atrophic gastritis (2, 9). This information has led Bernhardt and associates to comment that parietal cell antibody fundamentally reflects atrophic gastritis and is not specifically related to pernicious anemia. They concluded that the test, although useful in screening patients for early asymptomatic atrophic gastritis is of limited value in the differential diagnosis of anemias (10).

We cannot agree with this conclusion for any test that is positive in nine out of ten patients with pernicious anemia is diagnostically very useful when other conditions often associated with such seropositivity can be excluded. Furthermore a negative result is of even greater significance and should cast serious doubt on the diagnosis of uncomplicated Addisonian pernicious anemia.

Irvine et al recently demonstrated that the quantity of intrinsic factor in gastric juice from patients with pernicious anemia is clearly less than that in patients with atrophic gastritis who in turn are deficient as compared with normal individuals (4). They suggested that patients at special risk be screened initially for parietal cell antibody and if positive have gastric acid determinations. He further suggested that any histamine stimulated specimen of gastric juice shown to be de-

void of acid should be submitted to qualitative intrinsic factor analysis

In the case reported here, our failure to demonstrate antibodies to parietal cells and intrinsic factor was the first clue leading to a reevaluation of the patient's initial diagnosis. Because of the subsequent demonstration of vitamin B₁₂ malabsorption it was necessary to study the patient's capacity to produce intrinsic factor. Like many individuals with atrophic gastritis, however, he secreted only a small amount of gastric juice, and thus immunoassay was the only feasible investigative method. Finally having documented by this method the presence of intrinsic factor, and with additional knowledge of the absence of antibodies to parietal cells or intrinsic factor, we are led to speculate that this is not a fortuitous association of Addisonian pernicious anemia with nontropical sprue, but rather an instance of atrophic gastritis causally related to the intestinal lesion. Wintröbe has stated that intrinsic factor could not be demonstrated in the gastric secretion of a few patients with sprue and that as many as 30% of patients with sprue have no free gastric acid (11). Our patient is currently being treated with a gluten free diet and will be observed for signs of gastric as well as intestinal improvement.

Summary

The unusual coincidence of vitamin B₁₂ and folic acid deficiency associated with both nontropical sprue and atrophic gastritis, is reported in an elderly Danish man. Although history, physical examination and hematology

studies suggested a diagnosis of Addisonian pernicious anemia, the failure to find antibodies to parietal cell antigen led to further investigations, including gastric and jejunal biopsies and estimation of intrinsic factor secretion, which established the primary disease as nontropical sprue. The value of immunologic methods in distinguishing Addisonian pernicious anemia due to a genetically determined deficiency of intrinsic factor from other forms of megaloblastic anemia is emphasized.

Acknowledgement

We are indebted to Dr. Lionel Head for performing the biopsies on this patient and to Dr. Keith Taylor for analyses of serum antibody to intrinsic factor and of intrinsic factor in the gastric juice.

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Megaloblastic Anaemia developing during treatment of Epilepsy

By ERIK KIORBOE & CLAUS MUNK PLUM

A variety of haematological changes may appear during anticonvulsive therapy. For example cases of aplastic anaemia and granulocytopenia have been observed during treatment with mephenetoin (VFV) (21 a)

Cases of megaloblastic anaemia have been reported so far unexplained. The anticonvulsive drugs suspected as possible causes of megaloblastic anaemia are phenytoinum (INL VLN) (1 2 3 4 7 11 17 18 26 29 32 and 44) primidonum (INV NFV) (3 4 13 15 29 and 33) amobarbitalum (INV) and secobarbitalum (INV) (19) phenobarbital (INV) (1a) and combination of phenobarbital and phenytoin (20 22). Barbiturates are also cited as causing the macrocytosis so often observed during the treatment of epileptic patients. The macrocytosis anisocytosis and other haematological features often appear without any signs of anaemia.

Thus Hawkins & Meynell (18) report that macrocytosis is found in about 4 per cent of patients receiving

treatment with phenytoin+phenobarbital in about 34 per cent treated with phenobarbital but only in 2a per cent treated with phenytoin alone.

The same authors have examined the vitamin B₁₂ content of serum in patients with macrocytosis without anaemia. They find that while their controls (22 persons) had a B₁₂ content corresponding to 210 µg/ml (105—385) a patient group examined had a mean serum content of 17a µg/ml. Six patients were then given 100 µg of cyanocobalaminum (INV) weekly for a period of two months without any demonstrable change in the blood picture.

Folic acid absorption from the intestinal canal and excretion of parenterally given folic acid were normal. After the treatment with cyanocobalaminum 20 mg folic acid were given perorally daily resulting in a rapid disappearance of the macrocytosis.

Chanarin *et al* (8) examined the clearance of intravenously injected folic acid in 8 patients with megaloblastic

Review of cases from the literature

	sex	age	Duration of treatment and drugs	Gastric function	Drugs with duration	Treatment and effect
Badenoch (1954)	f	22	Phenytoin Na Phenobarbital	achylia		B ₁₂
	f	17	Phenytoin Na Phenobarbital			folie acid
Hawkin & Meynell (1954)	m	52	Phenytoin Na 13 years	free acid		folie acid
Chalmers & Boleimer (1954)	m	32	Phenytoin 5 years Phenobarbital 5 years Primidone 2 months	free acid		folie acid
	f	47	Phenytoin			folie acid
Rhind & Varadi (1954)	f	10	Phenobarbital Phenytoin	achylia	B ₁₂	folie acid
Webster (1954)	f	23	Phenytoin	free acid		folie acid
Berlyne I evone McGivhan (1955)	m	28	Phenytoin Phenobarbital	free acid	+	B ₁₂
	m	26	Phenytoin Phenobarbital Primidone	free acid		folie acid
	m	32	Phenytoin Phenobarbital	free acid		B ₁₂ — no effect
Bylin & Iorshaw (1955)	f	28	Phenytoin Phenobarbital	free acid	+	folie acid
	f	27	Phenytoin Phenobarbital	free acid	+	B ₁₂
	f	28	Phenytoin Phenobarbital	free acid	+	folie acid
Vaishnavi (1955)	f	28	Phenytoin Phenobarbital	free acid		B ₁₂ +folie acid
	f	23	Phenytoin Phenobarbital	free acid		folie acid
Cordwood	f	41	Phenobarbital Primidone	free acid	+	folie acid
	m	17	Phenytoin Phenobarbital	free acid		folie acid

Review of cases from the literature

	sex	age	Duration of treatment and drugs	Gastric function	Drugs with drawn	therapy and effect
Badenoch (1954)	f	22	Phenytoin Na Phenobarbital	achylia		B ₁₂
	f	17	Phenytoin Na Phenobarbital	free acid		folic acid
Hawkin & Mcynell (1954)	m	52	Phenytoin Na	free acid		folic acid
Chalmers & Bohemer (1954)	m	32	Phenytoin Phenobarbital Primidone 2 months	free acid		folic acid
	f	47	Phenytoin	achylia		B ₁₂
Rlund & Varadi (1954)	f	19	Phenobarbital Phenytoin	free acid		folic acid
Webster (1954)	f	23	Phenytoin	free acid	+	B ₁₂
Berlyne I evene McGlashan (1955)	m	28	Phenytoin Phenobarbital	free acid		folic acid
	m	26	Phenytoin Phenobarbital Primidone	free acid		B ₁₂ — no effect
	m	32	Phenytoin Phenobarbital	free acid	+	folic acid
	f	28	Phenytoin Phenobarbital	free acid	+	B ₁₂
	f	27	Phenobarbital	free acid	+	folic acid
Vaughan (1955)	f	28	Phenytoin Phenobarbital	free acid		B ₁₂ + folic acid
	f	23	Phenytoin Phenobarbital	free acid		folic acid
Girdwood	f	33	Phenobarbital Phenytoin	free acid	+	folic acid

Review of cases Continued

	sex	age	Duration of treatment and drugs	Gastric function	Drugs with drawn	therapy and effect
Kidd & Mollin (1957)	f	41	1 phenobarbital Phenytoin	free acid		(B ₁) folic acid
	f	34	1 phenobarbital 1 phenytoin 1 rimidonium	many years many years 2 years 18 months		
Bickler & Matzold (1957)	f	36	1 phenobarbital Mephentoin 1 rimidonium	free acid		died before treatment
Stokes & Fortne (1958)	m	16	1 phenytoin 1 phenytoin	free acid	+	(B ₁₀) folic acid folic acid
Gorlin (1958)	f	32	1 phenytoin 1 rimidonium	free acid		folic acid
Ives (1961)	f	36	1 phenobarbital 1 phenytoin Primidone	free acid	+	folic acid (B ₁₀) folic acid (B ₁₂)
	m	34	1 phenobarbital 1 phenytoin	free acid		folic acid (B ₁₀) folic acid (B ₁₂)
	f	46	1 phenobarbital 1 phenytoin 1 rimidonium	achylia	++	folic acid (B ₁₂)
	f	34	1 phenobarbital 1 phenytoin 1 rimidonium	free acid	++	folic acid (B ₁₂)
	f	33	1 phenytoin 1 rimidonium	free acid	++	folic acid
	f	31	1 phenobarbital 1 phenytoin	free acid	+	(B ₁₀) folic acid (B ₁₀) folic acid (B ₁₀) folic acid (B ₁₀) folic acid (B ₁₂)
	m	5	1 phenobarbital 1 phenytoin	free acid	+	(B ₁₀) folic acid (B ₁₂)
Isner (1963)	f	38	1 phenobarbital 1 phenytoin	free acid	+	(B ₁₀) folic acid (B ₁₂)

blastic anaemia following or resulting from treatment with anti epileptic agents. They found that three of the patients had a normal clearance while the remaining five patients had a very fast clearance. The interpretation of the latter result is that the five patients have probably had a reduced folic acid content in their tissues while the three patients have had a normal values of folic acid. The authors consider that the origin of the anaemia is an interference with the utilization of folic acid in the tissues.

Druskin *et al* (10) report a case of megaloblastic anaemia developing during anti convulsive treatment with a normal diet containing presumably adequate amounts of folic acid. If a daily supplement of 20 μ g of pure folic acid was given the desired effect was obtained. The authors interpret this as a sign that the organism is unable to utilize the folic acid ingested in food.

In a normal diet folic acid is present in a polymerized bound form as a polyglutamate. In the contents of the normal intestine a depolymerizing enzyme is found — conjugase — which is able to convert polyglutamate into free folic acid. On the assumption that the anti epileptic drugs administered have an inhibitory effect on this conversion the organism will not be presented with adequate supplies of folic acid. This does not occur until pure folic acid is administered.

Some investigators consider that this microcytosis appearing during the treatment of epilepsy is a very early stage of megaloblastic anaemia.

Among patients with epilepsy the percentage of those who have macrocytosis and subsequently develop megaloblastic anaemia is not known.

Christenson *et al* (9) showed that if primidone was withdrawn in a patient with megaloblastic anaemia remission resulted. This must be regarded as support for the argument that primidone is the direct cause of the anaemia as there has been no lack of folic acid in the tissues.

As already mentioned Hawkins *et Meynell* (18) examined the part played by vitamin B₁₂ in megaloblastic anaemia. They showed that the mean serum content was about 40 μ g/ml below normal. Kidd *et al* (21) find values corresponding to 40—50 μ g/ml in two patients while the lowest normal value was 100 μ g/ml.

Badenoch (1) Chalmers *et Bohemer* (7) and Chanarin (8) quote normal amounts of the vitamin B₁₂. According to the literature (1, 7, 8, 21) various vitamin B₁₂ tests such as absorption uptake in the liver, excretion in the faeces and clearance all appear to give results which lie within normal limits.

The studies of Hawkins *et Meynell* (18) were made on patients on normal diet. Kidd *et al* (21) on the other hand found a rise of up to 48% in the reticulocyte count after the ingestion of 100 μ g of vitamin B₁₂. Hawkins *et Meynell* (17) found a rise of 14% in the reticulocyte count but no effect on the amount of haemoglobin.

There have been very varying results from a number of similar studies. It is very difficult to evaluate some of

them, however, as the patient has either had a transfusion (34), or there has been a change in anticonvulsive therapy (27), simultaneously with the vitamin B₁₂ treatment

Patients with megaloblastic anaemia resulting from treatment with anti epileptic drugs usually all show free acid in the gastric juice. If achylia is found, true pernicious anaemia must be considered to be present

The anaemia usually develops quite slowly. Bone marrow studies show a picture which is typical for pernicious anaemia, with a greatly increased number of megaloblasts. The myelopoiesis shows a decided shift to the left with asynchronous maturation. Poikilocytosis, anisocytosis and a rise in the number of hypersegmented granulocytes are observed in the peripheral blood. Serum vitamin B₁₂ is reduced in most cases, values down to 40 µg/ml often being observed. The folic acid content on the other hand is usually normal. The serum iron is sometimes elevated, sometimes normal, but constitutes up to 60–80 per cent of the transferrin (TIBC) amount.

A sudden fall in serum iron may be observed during the treatment with folic acid as early as within 3–4 hours after start of treatment.

During the treatment the serum B₁₂ most often shows a slow rise. A typical reticulocyte crisis is seen even though the means used to combat the epilepsy continue to be employed during the treatment. The mechanism of this appearance of megaloblastic anaemia is unknown.

Chemically, there is a certain structural resemblance between folic acid, phenytoin, primidone and phenobarbital (14). This suggests the possibility of the competitive inhibition of the metabolic process normally associated with folic acid, but it is so far quite inexplicable why such a small proportion of the patients treated with these drugs should develop this type of anaemia.

In addition to the literature cited it should be mentioned that Galenby (13a) in a retrospective study of 37 pregnancies in epileptics receiving anticonvulsant therapy found no cases of megaloblastic anaemia. In 48 pregnancies receiving phenobarbital alone. In 7 pregnancies where phenytoin was used as well as phenobarbital there were 4 cases (one unconfirmed) of severe megaloblastic anaemia. Folic acid cured the anaemia in these cases although the anticonvulsant therapy was continued. Zachau Christiansen et al (35) examined 367 pregnancies with regard to the amount of folic acid in serum. They found decreased values in 31 per cent. Among these 367 patients 9 were suffering from epilepsy and in all these the amount of serum folic acid was decreased.

Present case

The patient is a 43 years old man who suffered from Huntington's chorea and epilepsy since the age of 28 years.

1st admission Koloniien Filadelfia 26.1.1955 to 3/2.1956 (case record no. 34036)

2nd admission Koloniien Filadelfia 1/2.1961 (case record no. 41377) for periods staying at a nursing home.

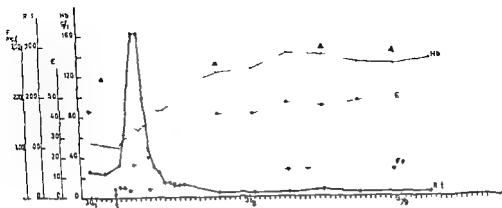


Fig 1 Showing some haematological data during the treatment of a case of megaloblastic anaemia during treatment with anticonvulsiva

- Serum iron ○ — ○ Erythrocytes
 ▲ Serum transferrin (TIBC) x — x Haemoglobin
 + — + Reticulocytes

Since 1956 the patient has been treated with phenytoinum and primidonium. Since 1961 phenytoinum mg 400 primidonium 750 mg. Since 1963 chlorpromazinum 100 mg has been given in order to control the choreoid movements. During the summer 1963 he developed clinical symptoms of anaemia and a haematological study on the 30 of July showed Hb 33 g/l erythrocyte count 129 million index of colour 1.37 Erythrocyte volume 180 ml MCV 140 mvl MCH 306 g/l Chlorpromazinum was withdrawn on the 31/7 and treatment commenced with folic acid mg 10 4 on the 4/8 Chlorpromazinum was readministered from 11/8 and a supplement of iron (Ferromyn Distral) was given from 13/10.

The remaining haematological data and the iron concentrations during treatment are seen in fig 1.

Gastric juice Free acid (Diagnex blue method) Histamine test meal + Congo reaction Examination of gastric juice for intrinsic factor (1/9) showed positive reaction (We are bringing our thanks to M. Schwartz M. A. S. Glæstrup for carrying out this analysis). Further data before the beginning of treatment with folic acid: Serum folic acid 2.8 0.012

µg/ml 0/10 0.075 µg/ml Serum B₁₂ 2.8 30 pg/ml 8/9 120 pg/l Serum transaminase normal serum electrolytes normal Thymol and Takata Ara reaction Normal Bilirubin 7.4 mg/ml serumprotein 39 g/l creatinine 6 mg/l osmotic reaction commencing haemolysis 0.3 % total haemolysis 0.33 % NaCl ESR 2.8 21 mm/hr 13/9 2 mm/hr

Iliac crest puncture 2/8: every cell rich marrow was extracted. Erythropoiesis completely dominated by megaloblasts and proerythroblast in all stages of development. Only a few normoblasts are seen. The number of mitoses is greatly increased. There are numerous "twin cells". Granulocytopoiesis: numerous "giant metamyelocytes". Increased number of mitoses among the myelocytes. Pronounced shift to the right. Increased number of eosinophilic cells. Lymphocytes few and normal. Monocytes few and normal. Plasmacells and haemocyctoblasts increased in number.

Diagnosis: Observation for pernicious anaemia or related type of anaemia. Marrow puncture was repeated after 4 weeks. The picture was like that normally found in cases of pernicious anaemia in remission and after 2 months the picture was quite normal.

Examination of faeces no positive benzedrine reaction no increased fat excretion

After 18 weeks therapy no symptoms of anaemia were present

Discussion

From the history and treatment this appears to be a case of megaloblastic anaemia, adequately treated with folic acid

The investigations carried out would seem to exclude the presence of idiopathic steatorrhoea and chronic disease of the liver. It is therefore reasonable to assume that the cause of the megaloblastic anaemia must be sought in the treatment of the epilepsy

Summary

A 43 year old man with Huntington chorea and epilepsy received treatment with phenytoin and phenobarbital for an extended period. The patient has been fatigued for some time, and the laboratory studies revealed a megaloblastic anaemia with nearly normal serum folic acid values. The condition was treated with folic acid, without withdrawal of the anti-epileptic drugs. Normal haemoglobin was obtained and the treatment of the epilepsy must be regarded as the actual etiological factor in the development of the megaloblastic anaemia in this patient.

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Über das Auftreten basophiler, auf den Erythrozyten aufgelagerter Korpuskel im Blute nach Vornahme einer Splenektomie

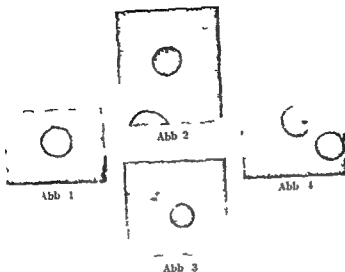
Von F. REIMANN

Im Verlaufe von Untersuchungen über das Auftreten von Howell Jolly'schen Körperchen (HJK) im Blute splenektomierter Patienten wurde die Beobachtung gemacht, dass in den fixierten und mit May Grünwald- und Giemsa-Lösung gefärbten Ausstrichpräparaten neben einer gewissen Anzahl von Erythrozyten mit grossen, tief gefärbten kreisrunden Körperchen im Inneren auch viele andere rote Blutzellen vorkamen, die nur kleine stecknadelkopfgrosse bis punktförmige, gut begrenzte und distinkt kolorierte Partikel enthielten. Während die grösseren Korpuskel die den HJK entsprechen, waren meist nur in Einzeln vorhanden, waren von den kleineren oft zwei, zuweilen drei und nur selten mehr als vier zu zählen.

Bei weiteren Untersuchungen ergab es sich, dass alle diese Gebilde sich auch mit basischen Vitalfarbstoffen, so vor allem mit Brillantkresylblau darstellen liessen. Sie nahmen diesen Farbstoff sogar recht gierig auf und kontrastierten bei richtiger Färbung

durch ihr purpurblaues Kolorit sehr deutlich mit dem gelblichen Ton des Haemoglobins in den intakten Erythrozyten. Gleichzeitig zeigte es sich, dass die Korpuskel, gross oder klein, nichts mit der vitalfärbbaren Substanz der Retikulozyten zu tun hatten und sich von ihr durch das Aussehen und ihren solitären Charakter unterschieden. Sie bildeten stets vereinzelte Gebilde, während die retikuläre Substanz den ganzen Erythrozyten durchzog.

Die HJK und ihre Eigenschaften sind allgemein bekannt. Ähnliche Gebilde wie die kleinen Partikel sind ebenfalls schon von verschiedener Seite beschrieben worden, wie aus den Handbüchern der Hämatologie (2) zu ersehen ist. Sie differierten aber von den geschilderten Gebilden in einer ganz besonderen Hinsicht. Es konnte nämlich einwandfrei festgestellt werden, dass die beobachteten Partikel sich nicht im Inneren der Erythrozyten befanden, sondern stets an der Aussenseite der Erythrozyten in ihrer



Bei allen Abbildungen Phasenmikroskop Öl Immersion ca 1000 fache Endvergrößerung Vitalfärbung mit Brillantkresylblau

- Abb 1 Nicht gefärbtes auf den Erythrozyten aufgelagertes Körperchen
- Abb 2 Aufgelagertes Körperchen das teilweise über die Zirkumferenz des Erythrozyten hervorragt
- Abb 3 Durch Strange an den Erythrozyten angeheftetes Körperchen
- Abb 4 Aufgelagertes Körperchen durch mechanische Einwirkung des Deckgläschens gegen das Zentrum des Erythrozyten gedrängt wobei die Erythrozytenmembran mitfolgt und eine Einkerbung an der roten Zelle erzeugt wird

Wird angelagert waren Dies traf auch für diejenigen Körperchen zu die innerhalb der flach ausgebreiteten Erythrozyten zu liegen schienen Diese Position war nur dadurch vorgezeichnet dass die wandständigen Körperchen in die roten Blutkörperchen projiziert wurden und wegen ihrer Dichte den Ländruck hervorrufen sich in den zentralen Partien der Zelle zu befinden

Beim Studium der Lokalisation der betreffenden Körperchen war das fixierte und mit Giemsa gefärbte Aus-

strichpräparat nicht sehr vorteilhaft doch konnte mit Hilfe von schiefer Beleuchtung und bei Anwendung des Phasenkontrastverfahrens bei geeignet situierten Körperchen ihre extracelluläre Lage deutlich wahrgenommen werden Viel geeigneter erwies sich in dieser Hinsicht die Vitalfärbung da mit ihrer Hilfe auch suspendierte Erythrozyten in noch lebendem Zustand von allen Seiten untersucht werden konnten

Am besten wurde zu diesem Zwecke ein kleiner Tropfen frisches Blutes auf einen

mit Brillantkresylblau gefarbenen Objektträger aufgebracht und nach Auflegen eines dünnen Deckgläschens durch vorsichtiges Ausstreichen verteilt. Durch leichtes Andrücken oder Emporheben des Deckgläschens an einer Ecke wurden geringe Strömungen im Präparat hervorgebracht, bei denen die Erythrozyten in Bewegung gerieten, ihre Lage wechselten und beim Zusammenstoß mit anderen roten Blutkörperchen sich um ihre Achse drehten und wendeten.

Schon bei Verwendung der normalen Ölimmersion konnte bei den vital gefarbenen Erythrozyten die externe Lage der beobachteten Korpuskel bemerkt werden. Bei den rotierenden und sich wendenden Blutkörperchen konnte recht eindrucksvoll verfolgt werden, wie die anscheinend zentral gelegenen Partikel plötzlich an den Rand des sich drehenden Erythrozyten gerieten und dann deutlich ihre aufgelagerte Position verrieten. Im Phasenbild zeigte es sich sogar häufig, dass die Partikel sich in einiger Entfernung von der äusseren Zirkumferenz der roten Blutkörperchen befanden und mit diesen durch feine Strange und Fäden verbunden waren. Zuweilen waren sogar nur frei flotierende Fäden sichtbar, in denen kein korpuskulares Gebilde angeheftet war.

Welche Bewandnis hatte es nun mit den beobachteten basophilen aufgelagerten Körperchen (b. r. k.)? Durch die besondere Lokalisation unterschieden sich die beobachteten Korpuskel von allen endocellulären Gebilden wie den von Heinz und von Schmauch (8) beschriebenen Innenkörpern der Erythrozyten und ebenso auch von den sogenannten Pappenheimer'schen Körperchen (5). Mit den letzteren wiesen sie zwar eine Reihe gleichartiger

Eigenschaften auf wie ihr Auftreten nach der Milzexstirpation, ihre Kleinheit, die multiple Zahl und die gleichen färberischen Eigenschaften Pappenheimer und Mitarbeiter (5) bezeichneten sie aber ausdrücklich als endocelluläre Elemente (erythrocytic inclusions).

Eine Beziehung zu den siderophilen Granula der Siderozyten war aus dem gleichen Grunde abzulehnen, da auch diese Granula ebenfalls nach der Splenektomie in vermehrter Zahl in den Erythrozyten nachzuweisen sind, intracellulär gelegen sind. Brüscke (1) stellt in seiner Monographie über den „Siderozyten“ die obige Forderung auf, dass nur solche Ausstrichpräparate verwendet werden, in denen extracellulär keine siderophilen Granula bzw. Kunstprodukte gefunden werden.

Kein Zusammenhang bestand auch mit den von Röhl (6) beschriebenen und später besonders von Jürgens und Schurer (3) genauer untersuchten „Randkörperchen“. Die letzteren färben sich zwar mit verschiedenen Vitalfarbstoffen, jedoch nicht mit Giemsa-Lösung. Das entgegengesetzte Verhalten zeigen die „Erythrokonten“ Schilling's (7), die deswegen und auch wegen ihrer stäbchenförmigen Gestalt von den b. r. k. zu unterscheiden sind.

Schliesslich konnte auch der Verdacht entkräftet werden, dass es sich bei den b. r. k. um parasitäre Elemente handelt, die nach Art der Bartonellen an den roten Zellen haften und die wie bei der Rattenbartonella erst nach der Milzexstirpation an den Erythrozyten in grosser Zahl in Erscheinung treten. Alle Blutkulturen blieben negativ und die Verabreichung von verschiedenen antibiotischen Präparaten in hoher Dosis und durch lange Zeit führte bei den untersuchten Fällen weder zu einem Verschwinden der aufgelagerten Körperchen noch selbst

zu einer Verminderung ihrer Zahl im übrigen ist hervorzuheben, dass auch von Pappenheimer und Mitarbeitern bei den von ihnen beschriebenen Körperchen in einen Befall der Erythrozyten mit Bakterien gedacht wurde eine Ansicht die sie später widerfallen liessen.

Die beobachteten aufgelagerten Körperchen waren also Gebilde von ganz eigener Art. Zum Verständnis ihrer Bedeutung ist zunächst hervorzuheben, dass sie bei Patienten gefunden wurden bei denen es sich um Fälle von Leberzirrhose und von Pfortaderthrombose mit grossem Milztumor und schwerer portaler Zytopenie handelte. Bei keinem der 10 untersuchten Patienten waren trotz sorgfältiger Prüfung die betreffenden Gebilde an den Erythrozyten vor der Splenektomie festzustellen obwohl in den meisten Fällen eine hochgradige Anämie bestand und im Knochenmark alle Zeichen einer starken regenerativen Hypertrophie und Hyperplasie des erythropoetischen Systems vorhanden waren. Nur nach der Milzentfernung und dies bereits in wenigen Tagen nach der Operation waren die Körperchen an den roten Zellen im kreisenden Blut nachzuweisen. Sie nahmen rasch an Häufigkeit zu und waren in einigen Wochen oft fast bei der Hälfte der zirkulierenden Blutkörperchen zu beobachten. Später verminderte sich gewöhnlich ihre Zahl und Grösse, doch verschwanden sie nie vollständig aus dem Blut und waren noch viele Jahre nach der Exstirpation der Milz in den roten Blutkörperchen festzustellen.

Die b k verhielten sich also in

dieser Hinsicht wie die H J K die ebenfalls erst nach der Splenektomie im Blut erscheinen und dann durch viele Jahre nachzuweisen sind. Auch in anderer Beziehung wie H B in der Irregularität der Form liegt eine gewisse Verwandtschaft vor. Die H J K können auch in Mehrzahl vorkommen. Nur in der Herkunft scheint ein wesentlicher Unterschied zu bestehen.

Die H J K werden ganz allgemein als Reste des Erythrozytenkernes angesehen. Diese Deutung liess sich aber nur schwer auf die b k übertragen. Die extracelluläre Lage, die multiple Vorkommen, die Kleinheit und die oft diametral entgegengesetzte Position der einzelnen Teilchen an einem Erythrozyten machten eine nukleäre Herkunft der b k recht wenig wahrscheinlich. Die letzteren Eigenschaften sprachen vielmehr für eine Auflagerung erythrozytenfremder Bestandteile an der Oberfläche der roten Blutkörperchen nach Art eines Coatings. Es konnte sich z B vermutungsweise um die Adsorption von Bestandteilen anderer Zellen und Kerne mit denen die roten Blutkörperchen bei ihrer Zirkulation im Organismus beider wurden oder aber auch um die Aufnahme von Resten der interzellulären Substanz aus dem Knochenmark handeln. Für das Vorhandensein eines allgemeinen Belags auf den Erythrozyten spricht auch die erwähnte Beobachtung im Phasenmikroskop von bewachsenen fadenförmigen oder strängartigen Auflagerungen an der Oberfläche der Erythrozyten im Blute nach der Milzexstirpation an denen keine Körperchen angeheftet waren.

mit Brillantkresviblau gefärbten Objektträger aufgebracht und nach Auflegen eines dünnen Deckgläschens durch vorsichtiges Ausstreichen verteilt. Durch leichtes Andrücken oder Emporheben des Deckgläschens an einer Ecke wurden geringe Strömungen im Präparat hervorgebracht, bei denen die Erythrozyten in Bewegung gerieten, ihre Lage wechselten und beim Zusammenstoß mit anderen roten Blutkörperchen sich um ihre Achse drehen und wendeten.

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La place actuelle dans la nosologie de la Cyanose Methemoglobinémique Héritaire, "C M H "

Par ANTOINE CODOURIS

Le but de ce travail est de présenter ici l'ensemble des résultats de nos recherches clinico-biologiques et génétiques sur cette curieuse nouvelle maladie génotypique du sang la "Cyanose Methemoglobinémique Héritaire" de les comparer avec les résultats obtenus par d'autres auteurs dans différents pays au cours des 19 dernières années et de fixer enfin la place définitive qu'elle doit occuper dans la nosologie des cyanoses.

Par "Methemoglobinémie" on entend certains états morbides dus à l'élévation au dessus de la normale du taux de la methemoglobine sanguine et qui cliniquement se manifestent par une cyanose tantôt primitive et permanente tantôt acquise secondaire et éphémère.

La methemoglobine existe normalement dans la proportion de 1 à 0.10 à 0.15 pour 100 cc³ de sang (Paul). Elle peut cependant s'élever dans les différents syndromes cyanotiques jusqu'au taux de 52.20 % (Codouris). Elle est toujours intraglobulaire et jamais plasmatique sauf dans les cas des pro-

cessus hémolytiques infections à anaérobies éclampsie hémoglobinurie paroxystique fièvre hémoglobinurique et ictère hémolytique (Bensky). La methemoglobine est un pigment brun différent de l'hémoglobine en ce que le fer y est à l'état trivalent. La methemoglobine (Met Hb) est un véritable oxyde dans lequel un atome d'oxygène est uni à un atome de fer. C'est un pigment inactif qui a perdu sa fonction respiratoire. Il s'agit d'un composé stable dont les éléments peuvent être libérés sous l'effet de réactions chimiques. La concentration normale de Met Hb se discute encore. Elle n'est probablement pas supérieure à 1.70 % (Wintrobe) de l'hémoglobine totale. Dès que le taux ci-haut est dépassé une cyanose particulière apparaît.

Le sang des sujets ainsi atteints de methemoglobinémie prend une couleur chocolat caractéristique due à la methemoglobine intraglobulaire. Le sérum par contre conserve sa couleur normale en raison de l'intraglobularité du pigment. La methemoglobine n'est pas toxique mais elle est incapable de

Das vollständige Fehlen der Korpuskel und Auflagerungen auf den roten Zellen vor der Milzentfernung und ihr promptes Auftreten nach der Splenektomie liessen auf die Fähigkeit der Milz schliessen, diese angelagerten „Verunreinigungen“ von den Erythrozyten zu entfernen. Die starke Deformation der roten Blutkörperchen, die sie bei der Passage durch die Poren der Milzsinus erleiden, wie dies so anschaulich in der Abbildung von Jung (4) demonstriert wird, liess eine mechanische „Waschfunktion“ der Milz als durchaus denkbar erscheinen. Das bruske Auftreten der aufgelagerten Gebilde nach der Exstirpation der Milz und das vollständige Fehlen vor der Operation mache es aber viel wahrscheinlicher, dass die „Reinigungsfähigkeit“ der Milz schon auch ausserhalb des Organs erfolgt und durch fernwirkende Stoffe im stromenden Blut und Knochenmark bewerkstelligt wird, die von der Milz erzeugt und abgegeben werden. Die „Säuberung“ der roten Blutkörperchen bleibt deswegen prompt und für die Dauer aus, wenn die Abgabe der „reinigenden“ Substanzen, seien sie fermentativer oder anderer Art, versiegt und ihre Quelle mit der operativen Entfernung des Organs für immer ausgeschaltet wird.

Zusammenfassung

Bei 10 Patienten mit Leberzirrhose bzw. Pfortaderthrombose, mit Milztumor und portaler Zytopenie waren nach der operativen Entfernung der Milz an vielen Erythrozyten Auflagerungen in Form von runden Korpuskeln und von fadenförmigen Strängen zu beobachten, die vor der

Splenektomie nicht vorhanden waren. Die Korpuskel kamen häufig in Mehrzahl vor, waren kleiner als die Howell-Jolly'schen Körperchen und färbten sich mit Giemsa und mit Vitalfarbstoffen wie Brillantkresylblau. Sie waren am besten im Phasenmikroskop zu beobachten und waren oft mit Strängen an der Erythrozytenoberfläche angeheftet. Die scheinbar zentrale Lage, die sie oft im Erythrozyten aufwiesen, war nur durch die Projektion ins Innere der Zelle hervorgerufen. Durch ihre Lage und die färberischen Eigenschaften unterschieden sie sich von anderen körperlichen Gebilden in den Erythrozyten.

Es wird nun angenommen, dass es sich um Auflagerungen erythrozytenfremder Substanzen handelt, die aus der Zirkulation oder aus dem Knochenmark stammen und die normalerweise bei der Passage durch die Milz entfernt oder durch besondere lytische Stoffe, die von der Milz erzeugt werden, im Kreislauf abgelöst werden.

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sa connaissance et à son individualité on ont fait beaucoup d'assez nombreux auteurs parmi lesquels le Professeur Codoun s d'Athènes et ses collaborateurs Loucatos et Louis des ont certainement apporté la plus grande contribution. Mais en Novembre 1916 Codounis et ses collaborateurs grâce à d'importants travaux nous renseignent définitivement sur le mode de transmission de la cyanose méthémoglobinémique héréditaire rapportant 11 nouveaux cas appartenant à une même famille de 103 membres les "Astochlari" relatés sur 5 fratries dont 8 branches. L'étude approfondie de l'arbre généalogique de cette famille poursuivie par les auteurs malgré de nombreuses difficultés leur permet de démontrer pour la première fois de façon incontestable le caractère héréditaire et familial et la nature génotypique de la maladie. Ces ras constituent des exemples indiscutables de transmission héréditaire de la tare morbide comme caractère dominant selon les lois Mendéliennes.

Depuis lors nos conclusions sur la CMH sont faibles que recueillies la confirmation d'autres auteurs également comme fait la liste au début le chercheurs qui se sont occupés de la question après nous en publiant les cas isolés ou des arbres généalogiques.

Ben s Coultas et Coll 1914 King et Coll 1914 Gelson et Harrison 1914 Horie et Wier arbre (généalogique complet 1918) Velluz et Lironnet 1918 Lutenbacher et al 1919 Elter et Price 1919 Codounis et al s l'arbre gén de Melanarides 1919 Zaccarias et al arbres gén 1920 Balza et Sugarni arbres gén canaliculés (croisés et coll) 1921 Breaker et al 1921 Wurtz et al coll 1923 Becknagel et Horie et al 1924 Claron et Bonat 1924 ou et E anguelou 1926 (et al) et coll 1927 J coll et coll 1928 Scott et coll 1929 1931 1932 Deprée et Hekman 1933 T w es et L e ll 1933 T w es et Morris 1932 E lhart et al 1932 Muller et coll 1963 Bethe et Hie nt 1964

Étude des cas de CMH en tant que maladie autonome du globe rouge juillet de 1916 à la fin de 1924 compris les 24 cas de nos deux arbres généalogiques.

Ces chiffres prouvent que la maladie n'est pas aussi rare qu'on semblait le croire jusqu'en 1916 ou on ne connaissait en tout que les 21 cas décrits de 1844 à 1914.

Si à ces 152 cas on ajoute les 21 cas anciens qui apparaissent aussi selon nous au cadre de la CMH on arrive au chiffre de 173 que nous avons pu recueillir dans la littérature internationale.

On a pu se rendre compte par ce qui précède que la Cyanose Méthémoglobinémique Héréditaire attiré depuis 1914 l'attention de plus en plus croissante du monde médical. Les cas de CMH publiés sont multipliés de plus les communications que nous avons faites.

Beaucoup d'auteurs en publiant des cas isolés ou des arbres généalogiques complets de CMH ont confirmé de façon incontestable nos conceptions et nos recherches. Certains décrivent même la CMH comme "Maladie de Codounis" (J Bernard De Minas Muzaire Benhamdoud Wolfgang Hie nt Townes etc).

Aujourd'hui l'indivisibilité de la Cyanose méthémoglobinémique héréditaire comme maladie autonome bien définie ne laisse plus aucune doute. La conviction du monde médical presque unanime sur nos conceptions et recherches concernant cette curieuse maladie héréditaire du globe rouge est telle que deux de nos études d'ensemble sur la CMH parues dans le "Sang" 1911 et dans la "Semaine des Hôpitaux de Paris" 1922 ont obtenu un des premiers prix attribués par le III^e Congrès de l'Union Internationale de la Presse Médicale (Tome 194) aux meilleurs travaux originaux publiés en 1922 et 1923 dans les revues médicales chirurgicales appartenant au groupe de l'Union Internationale de la Presse Médicale.

La place importante que nous permettaient pas d'entrer ici sur l'étude spéciale de la génétique du syndrome et de l'étiologie que etc de l'affectation pour lesquelles d'ailleurs il y a accord unanime de tous les auteurs qui se sont occupés de la question nous en donnerons au lecteur de se rapporter d'une part au travail présenté au I^{er} Congrès International de Génétique Humaine 1924 et l'autre part à notre étude générale sur "Détermination génétique"

fournir de l'oxygène aux tissus Les symptômes de la méthémoglobinémie sont fonction de l'intensité avec laquelle la méthémoglobine remplace l'oxyhémoglobine

A l'heure actuelle deux groupes de cyanoses, dues à la méthémoglobinémie, ont retenu l'attention d'un très grand nombre de chercheurs et de cliniciens

1 les cyanoses primitives constituées par des stables et permanentes dans lesquelles les sujets atteints naissent cyanotiques, sans cause apparente Le type représentatif de telles cyanoses est la "Cyanose Méthémoglobinémique Héritaire" individualisée par nous en 1946, maladie génotypique du globule rouge bien défini, présentant tant le caractère congénital que le caractère familial

2 les cyanoses acquises secondaires et éphémères par intoxication exogène ou endogène dans le cadre desquelles sont comprises les cyanoses médicamenteuses les cyanoses entérogènes et les cyanoses par absorption d'eau de puits très riche en nitrites

Qu'il nous soit permis de ne donner ici qu'un résumé succinct sur ce qui concerne exclusivement la "Cyanose Méthémoglobinémique Héritaire", renvoyant le lecteur en ce qui concerne particulièrement la méthémoglobinémie acquise pour tous détails à notre description générale "Die Methämoglobinämie" parue dans le 3^{me} volume du Handbuch der gesamten Hämatologie, (Urban Schwarzenberg München Berlin 1960 P 710—746)

On y trouvera également une bibliographie complète sur les cyanoses jusqu'en 1956

Historique

L'histoire de la maladie est marquée par deux étapes

La première remonte à 1844 date à laquelle J rançois décrit le premier cas belge de cyanose sans cardiopathie et se termine un siècle plus tard en 1946 avec le remarquable mémoire de Sievers et Ryon recueillant dans la littérature les seuls 19 cas isolés alors connus y compris leur propre cas et les deux cas de Lian et ses collaborateurs

Au cours de cette première période la maladie était considérée comme se présentant rarement et exceptionnellement

Les 21 cas publiés jusqu'alors y compris celui de Graybiel (1940) et celui de Barcroft et ses collaborateurs (1946) qui ne figuraient pas sur la liste des auteurs précités étaient désignés dans la littérature médicale et dans les Traités d'hématologie sous des appellations diverses méthémoglobinémie idiopathique méthémoglobinémie congénitale ou encore lorsque plusieurs membres d'une même famille étaient atteints méthémoglobinémie familiale ou encore méthémoglobinémie intraglobulaire chronique ou encore enfin cyanose congénitale et familiale etc

La seconde étape commence en 1946 avec la publication de notre premier arbre généalogique de C.M.H. de la famille cyanotique des "Vastochilari" qui pour la première fois a fourni la preuve formelle de l'hérédité de l'affection et nous a permis de compléter l'étude clinico-biologique de cette affection

A Codouris qui la décrit sous le nom de cyanose méthémoglobinémique héréditaire lui a depuis en 1946 consacré d'importants travaux Il a fixé ses limites nosologiques et reconnu sa transmission" écrit le Professeur J Bernard (1952) dans son remarquable traité sur les maladies du sang

De son côté Deminas dans sa thèse (Paris 1951) intitulée "Contribution à l'étude de la Cyanose Méthémoglobinémique Héritaire "Maladie de Codouris" écrit entre autres "A

Le symptôme humoral pathognomonique de cette affection est la présence constante de la méthémoglobine intracellulaire dans le sang des malades

Le terme de "Cyanose Méthémoglobinemique Hériditaire" que nous avons proposé des 1946 et qui des labord affirme les trois caractères de cette anomalie sanguine a) la cyanose, symptôme clinique dominant b) la méthémoglobinemie : caractère humoral spectroscopique pathognomonique c) la nature héréditaire de l'affection, dans laquelle nous retrouvons tant le caractère familial que congénital, est croyons nous le plus indiqué et devrait remplacer définitivement dans la terminologie médicale les appellations de cyanose congénitale ou familiale ou de cyanose méthémoglobinemique idiopathique intraglobulaire etc qui semment la confusion dans l'étude en général des cyanoses. Même l'appellation "Hereditary Methaemoglobinemia" qui figure dans certains derniers travaux de la "littérature internationale ne couvre que l'hérédité et le signe pathognomonique humoral de la méthémoglobinemie. Il manque cependant à cette appellation le signe capital clinique qui est "la cyanose"

C'est pourquoi nous insistons une fois de plus sur notre opinion que le terme le plus indiqué est celui de la Cyanose Méthémoglobinemique Hériditaire

Tous les cas qui figurent dans la littérature sous l'une des diverses appellations précitées ne sont en effet que des cas de CMH dont les arbres généalogiques n'ont pas été constitués chose évidemment pas toujours facile

Avec la dénomination de CMH, le diagnostic différentiel avec les trois autres groupes des cyanoses classiques par anoxémie par mélange des sang artériel et veineux et par polycythémie devient plus aisé étant donné que chez eux la méthémoglobinemie fait défaut

Le terme de CMH évoque enfin des labord dans l'esprit du clinicien le diagnostic différentiel avec les cyanoses du deuxième groupe par méthémoglobinemie c à dire les cyanoses acquises par intoxication médicamenteuse ou enterogène dans le spectre du sang des patients desquels on trouve soit la bande de méthémoglobine soit celle de sulfohémoglobine

Ces cyanoses toutefois sont secondaires éphémères et intermittentes de guérison immédiate aussitôt que la cause étiologique disparaît (l'intoxication exogène ou endogène)

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IIIe Volume du "Handbuch der Gesamten Hämatologie" (1960) Nous insisterons seulement un peu sur ce qui concerne la pathogénie de l'affection vue de l'importance de certains travaux parus dernièrement sur ce sujet et nous passons ensuite aux conclusions générales

Pathogénie

De toutes les hypothèses émises jusqu'ici sur ce sujet, il nous semble que deux sont à retenir

1 celle de Cox et Wendel qui pensent que la réduction de la méthémoglobine est, en grande partie du moins, en rapport avec la fonction des enzymes contenues dans les globules rouges ou avec leur absence

2 celle de Sievers et Ryon basée sur l'absence des systèmes reducteurs des globules rouges

Nous penchons plutôt pour la première de ces deux hypothèses

Nous pensons que les magnifiques recherches de Scott et ses collaborateurs, de Townes et ses collaborateurs ainsi que celles de Muller et ses collaborateurs, vont éclaircir à ce sujet bien des points encore obscurs. Ainsi De déficience enzymatique Diphorase Méthémoglobine A Hemoglobine M De déficience du NADPH₂ Méthémoglobine Reductase etc. voilà des chapitres qui sont à l'ordre du jour afin d'élucider le problème si passionnant de la pathogénie de la Méthémoglobinémie Héritaire

Quoi qu'il en soit, le fait essentiel demeure que la maladie est héréditaire et que le globule rouge acquiert par hérédité la tare morbide, le rendant incapable de réduire la méthémoglobine en hemoglobine

Conclusions

Il ne fait point de doute, après nos propres recherches suivies de celles d'autres auteurs au cours de ces dernières 19 années, que la "Cyanose Méthémoglobinémique Héritaire" (CMH) constitue dans la nosologie des cyanoses une entité morbide du globule rouge, autonome et bien définie

Certains auteurs ont bien voulu donner à cette entité morbide le nom de "Maladie de Codouris", "Codouris Disease", "Codouris'sche Krankheit", "Morbus Codouris"

Dans cette entité entrent toutes les cyanoses constitutionnelles congénitales, familiales et héréditaires. Ainsi aux trois groupes classiques de cyanoses par anoxémie, par mélange des sangs artériel et veineux, par polycythémie, il convient d'en ajouter un quatrième, celui de la "Cyanose Méthémoglobinémique Héritaire" (CMH) maladie se transmettant héréditairement au globule rouge et le rendant incapable de réduire la méthémoglobine en hemoglobine cyanose primitive et permanente

Cette maladie enzymatique, tout comme la maladie hémolytique congénitale, se transmet sans préférence tantôt par les femmes tantôt par les hommes en prédominance toutefois par les hommes et plutôt selon le caractère dominant que récessif

Bien que sur les 173 cas environ actuellement connus plus de 50 soient de provenance hellénique la maladie n'est pas particulièrement grecque. Répandue dans tous les pays, elle n'est pas aussi rare qu'on le supposait

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IV

ENDOCRINE SECTION

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Carcinoid Tumours

By W S PEART MD FRCP

One of the outstanding examples of the combination of clinical acumen and biochemical knowledge shown by Jan Waldenström has been the elucidation of the carcinoid syndrome. Following the early description by Cassidy (34) of the flush in two patients one said to have a carcinoma of the stomach the description of the clinical picture with right sided valvular lesions in the heart diarrhoea and flushing of various types was developed in studies by Waldenström and Ljunberg (33, 34) Thorson and his colleagues (30) and Björck Axen and Thorson (2). When Lembeck (13) found large quantities of 5 hydroxytryptamine (5 HT) in the tumours it was not long before Pernow and Waldenström (22) demonstrated excessive amounts of 5 HT in the blood and urine of such cases. Later it was found that most of the 5 HT even in these patients was bound in the platelets. One of the final excretion products 5 hydroxyindole acetic acid was demonstrated to be increased in such patients by the work of Page and his colleagues (16) and Sjördsma and Lidenfriend (26).

The direct proof that the tumour and its products were responsible for the clinical manifestations was best shown in a case reported by Thorson and his colleagues (31) where such a tumour developed in a teratoma of the ovary and removal was followed by a remission. At this time since much knowledge about the action of 5 HT had accumulated from the work of Erspamer (6) who had extracted enteramine from the intestine wall and had correlated it with the presence of the cells of Kultschitsky it was natural to attribute most of the symptoms and pathological changes to an excess of the active substance 5 HT. The demonstration that intravenous 5 HT could cause facial flush in both in normal subjects and in patients with carcinoid disease (17) added further weight to this view. Another twist to the biochemical story came when Waldenström Pernow and Silver (35) showed increased urinary excretion of histamine in many patients. They discussed the part that it might play in the syndrome and in some of their patients who had a peculiar geographical red

tially that some humoral influence attacked the endothelium and was removed in passage through the lungs it must not be forgotten that the left side may be affected in some patients (29). All efforts to convincingly demonstrate that \pm HT can produce such valvular disease on administration to experimental animals have failed. The histology of the valve shows the process of damage followed by repair and it would equally be arguable that an enzyme which attacked endothelial collagen could bring about this effect. The change is not peculiar to the valves and occurs in the lining of the atrium and ventricle as well as in pulmonary arteries.

Flushing

This most prominent feature of the syndrome was of course earlier thought to be due to release of \pm HT but our own investigations (18, 21, 24) cast considerable doubt on this. The first observation is that the typical spontaneous flush is most commonly bright orange to red and is a hot flush. This type is almost impossible to reproduce by a wide range of doses of \pm HT in patients with carcinoid disease. Our studies on the precipitation of flushes using small doses of adrenaline and noradrenaline showed that the most likely explanation of this flush which was exactly like the spontaneous flush was the release from the tumour and its metastases of a substance or substances which caused vasodilatation. In this

type of patient with a red warm flush direct examination of blood draining metastases did not show an increase in the amount of \pm HT in the plasma. Another strong point was that administration of \pm HT to these patients led to increased respiration and in some to marked dyspnoea which in most of these patients was absent even during the deepest flushes. However other types of patient were observed with blue to red flushes and a cooler skin who became wheezy during their flushing attacks. Direct examination of blood draining from this tumour revealed increased amounts of \pm HT in the blood during the attack (1) so that while this type of patient undoubtedly releases \pm HT which causes some of the symptoms the other type probably does not. At that time we felt for various reasons that histamine was unlikely to be directly implicated. Recently Oates and his colleagues (10) have shown increased amounts of bradykinin in the blood draining the tumour in various patients in whom a flush had been provoked by adrenaline or noradrenaline. They further extracted a kallikreinlike enzyme from the tumour tissue. Bradykinin intravenously causes a marked red warm flush and also lowers the blood pressure in many such patients. In many of the patients we studied the blood pressure was lowered during a flush but by no means always and the most reasonable explanation for the flush now seems to be bradykinin or a very similar substance.

flush with itching, it was suggested that there was either a general or local release of histamine. As far as the biochemical pathways in the tumour are concerned, apart from the presence of large amounts of 5-HT and the decarboxylase which seems to be a very universal enzyme and relatively non specific (32), Graham-Smith (8) has more recently demonstrated the enzyme which hydroxylates tryptophan in the tumour and has described some of its co factor requirements. Demonstrating how the study of a tumour can yield variable information bearing on normal physiology, he has shown the presence of this enzyme in those regions of the brain which are known to contain large amounts of 5-HT (9, 10).

It would now be well to turn to a critical examination of the relation between symptoms, biochemistry and pharmacology in this condition. From the work of Erspamer and others (6), it was known that 5-HT contracted many smooth muscles and is of course responsible for part of the vasoconstrictor action (serotonin) in serum. This had previously led to the extraction of 5-HT from serum by Rappaport Green and Page (23).

Diarrhoea

The stimulant action of 5-HT on intestinal smooth muscle led naturally to the belief that this was the cause of the severe diarrhoea and increased intestinal motility noted in these patients. This may well be so especially since the diarrhoea is one of the few

manifestations which can be abolished by use of 5-HT antagonists (20).

Oedema

Oedema is more difficult to understand, particularly the type which occurs on the face and hands during severe flushing episodes. It seems very unlikely to be due to 5-HT and some substance which increases capillary permeability must still be sought for.

Skin changes

Apart from the flushing, the next commonest skin manifestation is the permanent telangiectases, and these have not been satisfactorily explained. A most interesting patient was first described by Thorson and his colleagues (31) in whom pellagrous skin changes were reversed by the administration of niacin. There is a strong suggestion that some endogenous tryptophan is converted to niacin in the body and in an ill patient with diarrhoea and a poor intake of food the endogenous source might be of prime importance. The avidity of tumour cells for tryptophan might then cause deficiency of niacin and pellagra would result. The more general idea that tumour cells can successfully compete with other cells of the body for essential metabolites is of great importance in considering the metabolic effects of other cancers.

Valvular heart disease

While the high incidence of right-sided valvular disease suggested an

ginally have been biochemically isotopic, the process of becoming cancerous involves the preferential release of one of these originally possessed pathways. It would carry the implication that these pathways are only being suppressed and given the right conditions can be revealed. In considering the mechanisms behind differentiation of cells, gene suppression is considered to be of great importance (12-14) leaving the other gene controlled enzyme systems to develop preferentially. Imaginative study of these sorts of cancer cells may help to throw some light on this process. An alternative hypothesis which is to me much less attractive is that by chance when cancer affects a cell the biochemical derangement produced leads to the production every now and again of a pharmacologically active substance. In the first place the precise structure of some of these pharmacologically active substances in these tumours may shed light since obviously if for example the "vasopressin type" molecule produced by a tumour of the lung is different in some respect from the naturally occurring vasopressin some alteration in the ordinary biochemical pathway must be admitted and the first hypothesis will not stand on its own.

It can be seen from this line of thinking and work how much we owe to the original observations of I. M. Waldenström.

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A number of patients have been observed in recent years who have thrown further light on this syndrome. The biochemical aspects were first emphasised by Sundler and Snow (25) and the characteristic is that large amounts of 5-hydroxytryptophan are excreted in the urine. Normally it seems likely that the tumour cells contain enough oxidative enzymes to carry out the conversion of most of the 5 HT produced to 5 hydroxyindole acetic acid, so that only small amounts of 5 HT reach the urine and practically no 5 hydroxytryptophan. In this sort of patient, however, large amounts of both are present in urine. This suggests that the tumour cells are deficient in oxidative enzymes so that 5 hydroxytryptophan is released into the circulation and appears in the urine or is converted to 5 HT, which then itself appears in the urine. Williams and Sandler (37) pointed out that derivatives of the primitive foregut like the pancreas, bile ducts and bronchus give rise to this biochemical type of tumour. The absence of an enzymatic pathway is common in many cancers. It seems likely that the predominance of noradrenaline producing phaeochromocytomas is due to a deficiency of the methylating enzyme converting noradrenaline to adrenaline. Again such tumours are known which produce hydroxytyramine or even dopamine (7). In the case of the thyroid cancer cells seem incapable in most instances of iodinating the tyrosine fully so that the precursors mono and diiodotyrosine are produced (27, 28). In some tu-

mours it may therefore be a general rule that the final biochemical step is the only one which is removed and this raises the possibility that there is a strong association between cancer properties and a fairly orderly removal of a small part of the cell's biochemical potential. The relation between these two observations needs a lot more study. Another important point raised by these tumours is that, as in a patient studied at St Mary's Hospital (19), the primary tumour appeared as a pancreatic duct carcinoma and did not look like a conventional carcinoid tumour. Does this mean that a cell with a different histological appearance is really a modified carcinoid cell of the same embryological origin as the cells of Kulchitsky? This might well be the case in some tumours which have the same histological appearance as typical carcinoid tumours e.g. those occurring in the lung but many are described which are indistinguishable from cancers of particular organs such as oat cell carcinoma of the lung (11, 36). This brings the carcinoid syndrome into relation with all the other biochemical and pharmacological syndromes described in relation to cancers in different organs e.g. Cushing's syndrome in association with lung cancer, ADH secretion with lung cancer and phaeochromocytoma in association with thyroid carcinoma, to mention but a few. The nature of this association has emerged as one of the most fascinating problems in cancer. The most impressive hypothesis would be that since all cells must ori-

On the Prevalence and Incidence of Carcinoids in Malmö

By FOLKE LIVELL & KERSTIN WÄNSSON

Various authors have studied the incidence of carcinoid (1—7). Some of the figures given vary widely. This discrepancy may be due to differences in the composition of their series, i.e. heterogenous or selected, or to the use of different or unsatisfactory examination methods. It was therefore considered legitimate to present an analysis of the frequency of carcinoids found at biopsy and necropsy in the rather well defined population of Malmö which, as Waldenström (8) points out, is very well suited for frequency studies.

Material and methods

The material derives from the town of Malmö which has about 230 000 inhabitants. The town has only one general hospital which serves the entire population of the town. The necropsy frequency at the hospital is high (98—99 %) and about 60 % of all persons dying in the town are examined post mortem. The necropsies were performed at one department and with uniform methods. The gastrointestinal tract was always slit up, rinsed and

carefully examined. In cases with tumours a special search was made for metastases and in all such cases specimens were examined microscopically. The prevalence (defined according to recommendations of WHO "as the frequency of illnesses in existence during a defined period, whether they started before or during the period") was studied in necropsy material from the years 1959—1962.

The incidence (defined according to recommendation of WHO as "the frequency of illnesses commencing during a defined period or of persons becoming ill during the period") was studied in biopsy material from the years 1957—1962. Since all inhabitants of the town can receive hospital care free of charge, it may be assumed that practically all cases of carcinoid that produced symptoms or that were discovered incidentally at operation are included in the present material. The possibility of the disease having been discovered and treated surgically in some other hospital is negligible and can hardly influence the present analysis.

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On the Prevalence and Incidence of Carcinoids in Malmö

BY FOLKE LINELL & KERSTIN MÅNSSON

Various authors have studied the incidence of carcinoid (1—7). Some of the figures given vary widely. This discrepancy may be due to differences in the composition of their series i.e. heterogeneous or selected or to the use of different or unsatisfactory examination methods. It was therefore considered legitimate to present an analysis of the frequency of carcinoids found at biopsy and necropsy in the rather well defined population of Malmö which as Waldenström (8) points out is very well suited for frequency studies.

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Table 4 Metastases in carcinoids of gastrointestinal tract (14 cases)

Regional lymph nodes in mesentery	10 cases
Other lymph nodes	4
Liver	7 "
Spleen	1 "
Peritoneum	1
Skeleton	2 "
Adrenal	1 "
Pleura	1
Colon	1 "
Heart	1 "
Pancreas	1
Kidney	1
Lung	1
Thyroid	1

been performed 3 years previously for carcinoid of the caecum and according to the hospital records a typical carcinoid syndrome had been manifest before and had persisted after the operation. In three other cases carcinoids were found incidentally at operation. Diffuse abdominal pain had been noted in 2 cases and might have been due to carcinoid. In addition in 2 cases with extensive carcinoid growth symptoms of intestinal cancer had been observed. HIA examinations had of course not been performed in cases in which carcinoid had not been suspected.

In only 4 of the 23 cases could the carcinoid be regarded as the main cause of death. In all the other cases the carcinoid was an incidental finding. In 40% of the cases there was also some other malignant tumour, a frequency corresponding to that of malignant tumours in the entire necropsy material.

Biopsy material. This material dates from a six year period (1957—1962)

Table 5 Gastrointestinal carcinoid (operated series)

Sex	Age (yr)	Symptoms or main disease	Localisation of carcinoid
♀	80	Gastric symptoms	Stomach
♂	60	Colon cancer	Small intestine
♂	68	Colon cancer	Small intestine
♂	38	Symptoms of appendicitis	Small intestine
♂	65	Abdominal pain	Appendix
♀	40	Abdominal pain Ovarian endometriosis	Appendix
♀	40	Abdominal pain, Myoma of uterus	Appendix
♀	23	Symptoms of appendicitis	Appendix
♀	34	Symptoms of appendicitis	Appendix
♀	19	Symptoms of appendicitis	Appendix
♂	47	Rectal polyps	Rectum
♀	60	Sigmoid polyp Rig changes	Sigmoid

There were altogether 12 cases of gastrointestinal carcinoid (see Table 5) including as many as 6 cases of carcinoid of the appendix and 3 of the small intestine. One case was gastric cancer of carcinoid type. In another case a large tumour (14×10 cm) was found in one of the ovaries. The tumour was a teratoma with only carcinoid structure in the abundant connective tissue. This was the only tumour that had produced a carcinoid syndrome with flush. The symptoms ceased after operation when the HIA values also became normal. In the 12 cases with tumours of the gastrointestinal tract carcinoid may be regarded as the indication for opera-

Results

Necropsy material The prevalence of carcinoid is given in Table 1. All together 53 cases of gastrointestinal carcinoid were seen, which means a prevalence of 11%. The frequency of bronchial carcinoid was only 0.1%.

The age and sex distribution is given in Table 2. Carcinoid was never

Table 1 Prevalence of carcinoids in autopsy material

Year	Necropsies	Carcinoids	
		Gastrointestinal	Bronchial
1959	1094	7	0
1960	1194	12	3
1961	1220	15	1
1962	1320	19	1
Total	4828	53	5

Table 2 Age distribution of carcinoids in autopsy material

Age	Carcinoids			
	Gastrointestinal		Bronchial	
	♂	♀	♂	♀
40—49	2	—	—	—
50—59	—	2	—	1
60—69	18	7	—	—
70—79	10	2	—	2
80	9	3	1	1
Total	39	14	1	4

Table 3 Site of carcinoid (autopsy material)

Oesophagus+stomach	3
Duodenum	3
Jejunum	43
Meckels diverticle	1
Appendix	—
Caecum	3
Colon rectum	—
Total	53



Fig 1 Age distribution of autopsy material. The two first columns are perinatal mortality; the following are 5 year classes.

found below the age of 40 years. The necropsy material, however, belonged to higher age classes, as is apparent from Fig 1, which shows the age distribution for the year 1959. This distribution is representative of the entire material.

The sites of the gastrointestinal carcinoids are given in Table 3. Carcinoids of the small intestine were most common and were seen in 43 of the 53 cases. The absence of carcinoid of the appendix is noteworthy. About twenty-five per cent (13 of 53) of the carcinoids were multiple. In these cases the number of tumours varied between 2 and 14. Sometimes the carcinoids were crowded in a small area of the small intestine while in others they were scattered over a long segment.

Metastases were found in 25% of the cases of gastrointestinal carcinoid. The distribution of metastases is given in Table 4. Only 2 cases with multiple carcinoids had metastases.

Only one case of gastrointestinal carcinoid was diagnosed on clinical grounds. In that case, which had widespread metastases, operation had

port of such an assumption neither did the present material in which the frequency of malignant tumours was the same (about 40 % in the carcinoid material as in the entire necropsy material)

As in published series carcinoid of the small intestine was more common in men than in women in the present material

Knowledge of the incidence *i.e.* appearance of diseases in a population during a given period of time is of both theoretical and practical interest. The present series of operated cases lends itself well to an elucidation of the incidence because it stems from a fairly well defined population of about 230 000 inhabitants of a town where all the inhabitants have free medical treatment at a single general hospital. The town has no private hospitals. The number of gastrointestinal carcinoids discovered at operation was 12 in 6 years *i.e.* somewhat less than one case per 100 000 inhabitants per year. None of these operated patients had a carcinoid syndrome which agrees well with the opinion of Moertel et al. that the carcinoid syndrome is rare and seen in only a very small percentage of patients with carcinoid tumour. In the present biopsy series the carcinoid syndrome had been noted only once in 6 years and that was in a case of ovarian teratoma with carcinoid structures. It is extremely difficult to give exact figures for the incidence of the carcinoid syndrome. As a rough guess it might occur once or twice every 10 years in the population of Malmö (230 000 inhabitants).

Natural history of carcinoid. All investigations on record suggest that carcinoid is a tumour which develops and grows very slowly. The observations made in the present investigation argue for this assumption. The operated series includes fairly many relatively young persons and in the necropsy series small carcinoids were seen in the aged. It was noteworthy that in the operated series half of the carcinoids were seen in the appendix, none in the necropsy series. Ritchie (6) has drawn attention to this point previously and suggested that it might be due to the appendix not having been examined at necropsy. This does not apply to the present material but as mentioned above all figures given here must be regarded as minimum figures. There is no convincing evidence that carcinoid of the appendix occurs earlier in life than carcinoid of the small intestine. It appears more likely that carcinoid of the appendix is discovered more frequently in the lower age classes owing to the wide range of indications for operation for symptoms in the region of the appendix. It has also been claimed that carcinoids that grow in the narrow appendix produce clinical symptoms quicker than carcinoids of the small intestine. This appears to be a plausible explanation why carcinoids of the small intestine are often not discovered until post mortem because they probably develop very slowly and asymptotically for many years. As pointed out by Moertel et al. (5) on the basis of a large series of 28 cases of carcinoid followed up at the Mayo

tion in 7, while in 5 the tumour must be regarded as an incidental finding at operation

Discussion

The prevalence of gastrointestinal carcinoid in a consecutive necropsy series was found to be 53 (1.1%) of 4828 necropsies. As mentioned, the figure is difficult to compare with that found in other series. Differences in examination technique, age distribution, selective factors owing to varying frequency of necropsy and the like may strongly influence the prevalence found. The present material has the advantage that it was examined by uniform methods and at a hospital with a high necropsy frequency (98—99%). This may explain why the figure found was much higher than most of those given in previous publications. Thus Ritchie (6) found the frequency in a compilation of published necropsy series to vary between 0.14% and 0.34%. Donald (2) published a necropsy series from Boston. Of 18,846 consecutive necropsies at Mallory Institute gastrointestinal carcinoid had been found in 72 (0.38%). Of 7,915 consecutive necropsies at six different Boston hospitals gastrointestinal carcinoid has been found in 0.38% (30 cases). The similarity in frequency is remarkable. In a series of 14,852 necropsies at the Mayo Clinic Moertel et al. (5) found the frequency to be twice as high (0.65%). For a Swedish series of more than 10,000 necropsies Thorson (7) reported a frequency of 0.08%. The examination of

that material had most likely been very unsatisfactory and the gastrointestinal tract had rarely been slit up and rinsed. His figure is surely only about one fifteenth of what it should have been. The high figure found in the present material may be due in part to the high age of the necropsy series but surely mainly to the examination method. As pointed out by several investigators, intestinal tumours are readily overlooked at necropsy. This probably also happened in some of our cases so that the number found is probably a minimum number. The highest prevalence (1.36%) was found by Feyerter (3) in an especially meticulous search for intestinal tumours in 2500 autopsies. On comparison with other series there appears to be reason to claim that the highest frequencies are the truest. There is no reason to suspect overdiagnosis in the present investigation because all cases were verified microscopically.

The frequency (25%) of metastasis in the present material is difficult to compare with that in other series because in most of the latter no distinction was made between biopsy and necropsy specimens. The frequency of metastasis was probably low compared with that in other series in which several small carcinoids had probably been missed. Ritchie (6) gives values between 21% and 75%.

Several investigators, including Moertel et al. (3) supposed a relationship between carcinoid and other malignant tumours. Feyerter (4) and Thorson (7) found no evidence in sup-

Organ Specific Antibodies in Addison's Disease

By J. NIELSEN, M. SOBOG, P. HALBERG and K. BROCHNER MORTENSEN

Tuberculous adrenitis has become less common during the last decades whereas the relative incidence of so called idiopathic Addison's disease has increased and now it is the most common type (11, 13, 26).

In the discussion of the etiology and pathogenesis of idiopathic Addison's disease genetic factors have been considered but only few studies have been published (6, 24).

In recent years it has been suggested that autoimmune mechanisms might be of pathogenic importance for the development of idiopathic Addison's disease. This assumption is supported by a number of histological, serological, experimental and clinical observations.

(a) The adrenal cortex in idiopathic Addison's disease reveals signs of chronic adrenitis. Three histological changes are found viz. round cell infiltration, progressive fibrosis and epithelial changes consisting of atrophy and patchy hyperplasia (7, 9, 13, 20). These changes are very similar to those found in chronic thyroiditis and chronic sialoadenitis in which auto

immunity might be of pathogenic importance.

(b) Organ specific antibody against a cytoplasmic antigen in the adrenal cortical cells have been demonstrated in sera from patients with Addison's disease (1, 2, 3, 10, 18) by an immune fluorescence technique and by a complement fixation reaction. This antibody has been found with considerable frequency in sera from patients with idiopathic Addison's disease whereas it is very unusual in sera from patients with tuberculous Addison's disease (10).

(c) Experimental adrenitis similar to the one found in human idiopathic Addison's disease and circulating adrenal antibodies have been produced by the injection of autologous homologous and heterologous extracts of adrenals (1, 8, 19, 21, 28).

(d) Several reports have been published about the coexistence of chronic thyroiditis and Addison's disease (4, 5, 12, 22, 27). Thyroid antibodies are frequently found in sera from patients with Addison's disease (3, 16, 18, 7) and the ultimate result of Ha

clinic, these tumours grow and metastasize very slowly

Summary

The prevalence of carcinoids was studied in a representative 4 year necropsy series from Malmö (230,000 inhabitants) where about 60 % of all people dying in the town are necropsied in the hospital. The prevalence of carcinoid of the gastrointestinal tract was about 1.1 % (53 of 4828 necropsied), the corresponding figure for bronchial carcinoid being 0.1 %. Most of the carcinoids were found in the small intestine and none in the appendix. In 25 % of the cases the carcinoids were multiple and in 25 % they had metastasized. There were fewer cases with metastases among the multiple carcinoids (only 2 cases). All of the carcinoids except four were incidental findings.

The incidence of carcinoids was studied in a 6 year series of patients operated upon at Malmö general hospital which is the only hospital in the town. The incidence of carcinoid of the gastrointestinal tract was less than 1

per 100,000 inhabitants per year. Most of the cases were carcinoids of the appendix. The carcinoid syndrome which is very rare (and has been seen in only an extremely low per cent of all cases of carcinoid) was noted in a case of ovarian teratoma with carcinoid structures. The incidence of bronchial carcinoid was somewhat less than half of that of carcinoid of the gastrointestinal tract.

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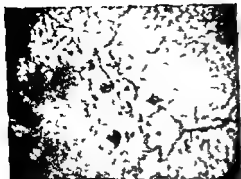


Fig 1 Infixed section of adrenal treated by immune fluorescence technique with a serum from a patient with Addison's disease. Well defined border between marrow without fluorescence and cortex with cytoplasmic fluorescence

guenon adrenal) is of a convenient size making it possible to obtain cross sections of the whole organ in which a well defined border between cortex and medulla is seen, thus making the results easy to read.

Microsomal thyroid antibody was demonstrated by an immune fluorescence technique as described by Holborrow *et al* (12). The microsomal thyroid antibody was titrated by a complement fixation reaction as described by Roitt & Doniach (21). Thyroglobulin antibody was demonstrated by means of thyroglobulin sensitized sheep cells from Bourroughs Wellcome & Co.

Results Tables 2-3 shows the results. Sera from 32 (50 per cent) of the total number of the patients with Addison's disease contained adrenal antibody.

Adrenal antibody was found in 66 per cent of sera from patients with idiopathic Addison's disease (group A), the incidence being the same in sera from male and female patients. Adrenal antibody was found in sera from two of the patients with Addi-

son's disease with known aetiology (group B) none of them had tuberculosis. Two of the patients in group C had adrenal antibody in their sera. One of them had had pulmonary tuberculosis 11 years before the onset of Addison's disease, the other patient had a calcified hilar lymph node. It is quite possible that Addison's disease in these cases was idiopathic and not due to tuberculous adrenalitis. None of the control sera (group D) contained adrenal antibody.

Thyroid antibodies were demonstrated in 42 (60 per cent) of all the patients.

The most common finding was a low titre of thyroglobulin antibody. The microsomal thyroid antibody was found in 27 of the patients with Addison's disease but not in sera from the control patients.

Thyroid antibodies were found in 27 (76 per cent) of the patients with idiopathic Addison's disease (group A). All high titres of both microsomal and thyroglobulin antibodies ($>16 >2000$ respectively) were found in this group. Among these patients were almost twice as many women as men. Thyroid antibodies were found in 15 (68 per cent) of the patients in group B+C but all the titres were low.

No correlation was found between the occurrence of thyroid antibodies and thyroid enlargements.

Discussion Although several observations indicate that autoimmune mechanisms may play a role in the development of Addison's disease the pathogenic importance of the circulat-

Table 1 Distribution of patients according to sex and aetiology

Sex	Total	Idiopathic Addison's disease (Group A)	Addison's disease with known aetiology (Group B)	Addison's disease and pulmonary tuberculosis (Group C)
Female	30	24	11	5
Male	30	24	9	2
Total	70	48 (68%)	10 (22%)	7 (10%)

shimo's thyroiditis, viz myxoedema has been reported coexisting with Addison's disease without signs of hypopituitarism (7, 4)

Own Investigations

In the following paper a report is given of adrenal and thyroid antibody studies in 70 sera from patients with Addison's disease

Material In cooperation with a number of the medical departments in Denmark, we have so far examined 70 sera from patients with unequivocal Addison's disease

Table 1 shows the material divided according to sex and aetiology

(Group A) 48 patients were considered having idiopathic Addison's disease since no aetiology could be found Half of the patients in this group were women, half were men Eleven of the patients, 9 women and 2 men, had goitres All the patients were euthyroid

(Group B) 15 patients had Addison's disease with a known etiology 12 of these patients had tuberculous adrenalitis This diagnosis was based on autopsy findings, adrenal calcifications by x-ray examination or by signs of haematogenic dissemina-

tion of tuberculosis 2 patients had tumor metastasis to the adrenal glands and in one patient the adrenals had been destroyed by panarteritis

(Group C) 7 patients had roentgenological signs of pulmonary tuberculosis In these patients tuberculosis may or may not be of aetiological importance for the development of Addison's disease Two of these patients both of them euthyroid women, had goitres

(Group D) Control sera were obtained from 29 women aged 15-70 years admitted to the hospital with gastric and duodenal ulcers, arteriosclerotic heart disease and minor mental disorders None of the patients presented signs or symptoms of thyroid or adrenal disease All of them had sedimentation rates of less than 10 mm/h

Methods Adrenal antibody have been demonstrated by an immune fluorescence technique as described by Blizzard et al (2) A typical finding in positive cases was a distinct fluorescence in all the layers of the adrenal cortex contrasting with the dark medulla (Fig 1) As previously stated by Blizzard & Hyle the antigen is organ specific but not specific of species Consequently guenon adrenal were used for the demonstration of adrenal antibodies because of the easy availability of guenons and since the

ing adrenal antibody is still unknown. Only one example is known of an organ antibody with a cytotoxic effect. A factor specifically toxic to cultured thyroid epithelial cells has been demonstrated in sera from patients with Hashimoto's disease and other thyroid disorders (17-20). This factor seems to be identical with the microsomal complement fixing thyroid antibody (10-14). A similar toxic effect has not been described for sera from patients with Addison's disease. No reports have been published about the possible importance of delayed type hypersensitivity for the development of chronic adrenitis.

The demonstration of the adrenal antibody may be of help in ascertaining whether tuberculosis is of pathogenic importance in a case of Addison's disease because the antibody was found in 66 per cent of sera from patients with idiopathic Addison's disease but until now it has been found in no cases of unequivocal tuberculous Addison's disease in our series.

The high incidence of thyroid antibodies in sera from patients with Addison's disease is in accordance with previous observations (4-7, 16). These findings correspond with the frequent occurrence of thyroiditis in Addison's disease.

Summary

Antibody against a cytoplasmic antigen in adrenal cortical cells was demonstrated in sera from 50 per cent of 70 patients with Addison's disease by means of immunofluorescence

technique. 60 per cent of the sera contained thyroid microsomal and thyroglobulin antibody. The adrenal antibody was found in 66 per cent of 48 sera from patients with idiopathic Addison's disease but it was not found in any case of unequivocal tuberculous Addison's disease. The adrenal antibody was demonstrated in no sera from individuals without Addison's disease.

Acknowledgement

We wish to thank the heads of the Danish medical departments for their help and cooperation in collecting the material.

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Table 2 Organ specific antibodies in sera from patients with idiopathic Addison's disease (Group A)

Sex	Number of Patients	Adrenal antibody	Thyroid antibodies			Adrenal and thyroid antibodies
			I Microsomal antibody	II Thyroglobulin antibody	I and/or II	
Female	24	16	13	14	17	11
Male	24	15	7	7	10	10
Total	48	31	20	21	27	21

Table 3 Organ specific antibodies in sera from patients with Addison's disease of known aetiology (Group B)

Sex	Number of Patients	Adrenal antibody	Thyroid antibodies			Adrenal and thyroid antibodies
			I Microsomal antibody	II Thyroglobulin antibody	I and/or II	
Female	8	1	1	2	3	0
Male	9	1	2	4	6	1
Total	15	2	3	6	9	1

Table 4 Organ-specific antibodies in sera from patients with Addison's disease and pulmonary tuberculosis (Group C)

Sex	Number of Patients	Adrenal antibody	Thyroid antibodies			Adrenal and thyroid antibodies
			I Microsomal antibody	II Thyroglobulin antibody	I and/or II	
Female	5	2	3	4	5	2
Male	2	0	1	0	1	0
Total	7	2	4	4	6	2

Table 5 Organ specific antibodies in sera from control persons (Group D)

Sex	Number of Patients	Adrenal antibody	Thyroid antibodies			Adrenal and thyroid antibodies
			I Microsomal antibody	II Thyroglobulin antibody	I and/or II	
Female	29	0	0	8	8	0

On the Adrenocortical Production of Sex Hormones in Gonadectomized Rats¹

By STIG KULLANDER

It has often been shown that the adrenal cortex produces steroid sex hormones and undergoes proliferative or tumorous changes in gonadectomized or old animals (for litt see Thung 1962). Frantz & Hirschbaum (1949) who studied various strains of gonadectomized mice observed that in some of the strains the pattern of the adrenocortical hormones was dominated by oestrogen in others by androgen. Wolley *et al* (1941) and Dickey & Wolley (1949) expressed the view that the adrenocortical activity after gonadectomy starts sooner in males than in females irrespective of the age of the animals at the time of gonadectomy.

Androgen and oestrogen are possibly produced simultaneously in gonadectomized mice with adrenocortical tumour or adrenocortical hyperplasia for female mice of the cc strain with adrenocortical tumours

after gonadectomy sometimes have a submandibular salivary gland of histologically male type — a sign of androgen stimulation — in association with signs of oestrogen stimulation in other organs (Wolley & Little 1945).

The effect of gonadectomy on the production of sex hormones and the formation of sex hormone producing adrenal tumours has not been studied so extensively in rats as in mice. It is generally believed that adrenocortical tumours are rare in rats (Houssay *et al* 1955, Cohen *et al* 1957, Iglesias *et al* 1958). Houssay *et al* (1954, 1955) and others have however, induced adrenocortical tumours in rats by gonadectomy. In rats of the Osborne Mendel strain adrenocortical tumours are common possibly because of a general endocrine imbalance in this strain since these animals often have co existing tumours of the adrenals, ovaries, mammary glands and pituitaries (Snell & Stewart 1959).

The growth of certain hormone dependent tumours such as mammary carcinoma and prostatic carcinoma in

¹ The animal operations were done in the Biolog. Dpt. Antoni v. Leeuwenhoekhuis in Amsterdam. Prof. O. Muhlbeck there kindly placed laboratory facilities to my disposal.

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Fig 1 Vaginal isograft in adrenal cortex of gonadectomized 2 month old male rat. Hix eosin $5\ \mu$. $\times 210$



Fig 2 Isograft of ventral prostate in adrenal cortex of gonadectomized 2 month old male rat. Hix eosin $5\ \mu$. $\times 700$

males killed at 2 months (Group I) this vaginal graft had formed a cyst (Fig 1). In both males and females the cysts contained some leucocytes but no cornified lamellae and the vaginal epithelium grew partly on its original stroma and partly on adrenocortical cells. In both the males and the females the vaginal graft in the adrenal cortex showed roughly the same picture of inactivity and the same picture as the actual vagina *in situ*. In both males and females the prostatic graft in the adrenal cortex showed roughly the same picture (Fig 2) resembling that of the actual prostatic gland *in situ* which showed signs of slight secretory activity of the epithelium with somewhat basally displaced nuclei indicating mild hormonal stimulation.

In the animals that were killed at 18 months after gonadectomy and which had had implants in the adrenals for 17 months (Group II a) the prostatic gland *in situ* showed only slight signs of stimulation. The vagina

in situ showed no signs of hormonal stimulation. The prostatic graft in the adrenal cortex of the males was only slightly stimulated (Fig 3) and the vaginal graft exhibited a picture of inactivity. The same findings were made in the female rats. On the other hand in 1 female rat in which one of the ovaries had been left *in situ* by mistake both the vaginal implant in the adrenal cortex and the vagina showed similar hormonal stimulation with the picture of pre oestrus.

In Group II b in which the grafts were implanted at 17 months and the animals killed at 18 months the males showed signs of increased androgen activity (compared with the animals in Group II a). They showed increased stimulation of the prostatic gland *in situ* and of the prostatic implant in the adrenal cortex (Fig 4). In addition the vaginal graft in the adrenal cortex showed slight mucification. Signs of slight increased androgen activity were also seen in the prostatic graft in the adrenal cortex of the fe

man can sometimes be arrested by extirpation of the adrenals in previously gonadectomized (castrated) patients. In these cases it is assumed that the operation includes removal of a "vicarious" source of sex hormone production (Brown *et al* 1959). It is not known whether oestrogens are the only hormones produced or whether they simply dominate the hormone pattern in gonadectomized women and whether androgens are the only or predominant hormones produced by gonadectomized men. Nor is it known whether the interval between gonadectomy and this hormone production varies with sex.

The purpose of the present investigation was to elucidate such questions experimentally. The production of oestrogen and androgen by the adrenal cortex in gonadectomized male and female rats of different ages was studied at varying intervals after gonadectomy. For this purpose small fragments of the prostatic gland and of vaginal mucosa were isografted to the adrenal cortex. The histological picture of the implants was later also compared with that of the donor organs *in situ*.

Material and methods

All of the rats used were of the homozygotic R strain. Gonadectomy was done under ether anaesthesia by midline abdominal incision at 3 weeks, 17 months or 30 months. At the same time isografts of the ventral prostatic gland and vaginal mucosa respectively from 3 week old donors were implanted in the right and left adrenal cortex respectively. Some animals gonadectomized at 3 weeks

did not receive the isografts until at 17 or 30 months.

Some of the animals were killed one month after implantation of the isografts while others were allowed to survive longer. The adrenals with the isografts and the prostatic glands or the vagina *in situ* were excised and fixed in 4% formalin, embedded in paraffin and sectioned (adrenals serially) and stained with haematoxylin-eosin.

In sections of the prostatic gland hormonally stimulated secretory activity was indicated by lightened endoplasmic reticulum of the epithelial cells (Harlin 1957). The height of the epithelium also was noted and used as a measure of secretory activity. In the vaginal sections the thickness of the epithelium and the presence of cornification or mucification were taken as indicators of hormonal influences.

All the animals were brought up in separate cages of one and the same size and on the same diet which consisted of pellets whole wheat once a week and tap water *ad libitum*. At death the animals were examined and careful search was made for neoplasms.

The material is summarized in Table 1.

Table 1 Experimental animals

Group	Number	Age at gonadectomy	Age at implantation of isografts	Age at death
I	5♂ + 5♀	3 w	3 w	2 m
IIa	5♂ + 5♀	3 w	3 w	18 m
IIb	5♂ + 5♀	3 w	17 m	18 m
IIc	5♂ + 5♀	17 m	17 m	18 m
IIIa	9♂ + 5♀	3 w	3 w	31 m
IIIb	5♂ + 3♀	3 w	30 m	31 m
IIIc	3♂ + 4♀	30 m	30 m	31 m
Total	37♂ + 32♀			

Results

All of the prostatic and vaginal grafts took well in the adrenal cortex. In all



Fig 7 Ventral prostate *in situ* in male rat with prostatic isograft implanted in adrenal cortex at 3 weeks in association with gonadectomy. Animal killed at 31 months. Htx eosin 5 μ . $\times 700$



Fig 8 Ventral prostate isograft in adrenal cortex of male rat. Animal gonadectomized and graft implanted at 3 weeks. Animal killed at 31 months. Htx eosin 5 μ . $\times 700$

nified lamellae were observed in the vaginal graft but the vaginal epithelium was low. The prostatic transplant was somewhat less stimulated than in the male animals.

In Group III a in which the grafts were implanted at 3 weeks in association with gonadectomy and the animals killed at 31 months the prostatic gland *in situ* (Fig 7) showed signs of stimulation with relatively high secretorily active epithelium compared with corresponding animals but killed at 18 months. At the end of this period the vagina *in situ* showed only insignificant activity of the epithelium.

In male rats the prostatic graft (Fig 8) was much more stimulated than in the animals killed one year younger while the vaginal graft still showed no signs of hormonal response at all. In the females of this group both the prostatic graft and the vaginal graft showed insignificant stimulation.

One of the males in this group had an adrenomedullary tumour but nei-



Fig 9 Ventral prostatic isograft implanted in adrenal cortex of 30 month old male rat gonadectomized at 3 weeks and killed at 31 months. Htx eosin 5 μ . $\times 700$

ther the prostatic gland nor the grafts had been influenced by it.

In Group III b in which the grafts were not implanted until one month before the animals were killed the prostatic gland *in situ* and the vagina *in situ* showed somewhat higher hormonal activity than in the animals in which the grafts had been implanted at 3 weeks.

In the males the prostatic graft (Fig 9) was markedly stimulated and



Fig 3 Prostatic isograft implanted in adrenal cortex of male rat at gonadectomy at 3 weeks Animal killed at 18 months Htx eosin 5 μ $\times 700$



Fig 5 Prostatic gland of male rat gonadectomized at 17 months and killed at 18 months Htx eosin 5 μ $\times 700$

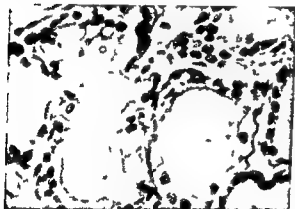


Fig 4 Prostatic isograft implanted in 17 month old male rat gonadectomized at 3 weeks and killed at 18 months Htx eosin 5 μ $\times 700$

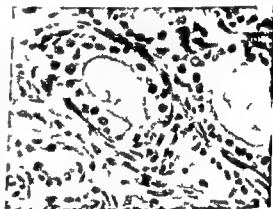


Fig 6 Prostatic isograft implanted in adrenal cortex of male rat in association with gonadectomy at 17 months Animal killed at 18 months Htx eosin 5 μ $\times 700$

male animals. In the female animals on the other hand neither the vagina *in situ* nor the vaginal graft in the adrenal cortex showed signs of stimulation.

In Group II c in which both gonadectomy and implantation of the isografts were performed at 17 months and the animals killed 1 month later the prostatic gland in the males showed distended spaces with low inactive

epithelium (Fig 5). The vagina *in situ* did not show such marked atrophy as in animals that had been gonadectomized for a long time — Group II a and b. In the prostatic graft in the adrenal cortex in these male animals signs of stimulation were seen with a higher lighter epithelium (Fig 6) than in the actual prostatic gland. In the vaginal graft however the epithelium was low. In the female rats cor

gonadectomized animals has been observed by previous workers. In young rats the adrenal is said to have a special andromimetic effect (Burtel & Greene 1939). In animals that were allowed to live 18 months the androgenic as well as the oestrogenic activity was minimal (Group II a) after 31 months (Group III a) the androgen production was increased but only in the males.

Gonadectomy at 17 or 30 months (Groups II and III c) seems to produce about the same activity in the transplants in the adrenal cortex soon after the operation as does gonadectomy before puberty (3 weeks of age).

The signs of increased stimulation observed in the prostatic grafts implanted in animals belonging to Group II and III b compared with II and III a were thus not due to increased reactivity in "younger" transplants but probably to the operations *per se* which has stimulated the steroid production by the adrenals. In oophorectomized women operative stress or the injection of ACTH increased the secretion of oestrogen suggesting a latent capacity of the adrenal cortex to produce oestrogens (Brown *et al* 1959). Administration of ACTH can however also increase the formation of androgen steroids in the human adrenal cortex (Soffer *et al* 1961). Cassner *et al* (1951) found injection of large doses of ACTH in cows to increase the androgen content of the blood in the adrenal veins.

The prostatic grafts sometimes showed more marked stimulation than the prostatic gland itself suggesting

that the androgenic substances produced in the adrenal cortex and capable of stimulating the prostate can exert to at least a part this effect without intermediate metabolism giving a strong local effect on the transplant. In the rat both transplanted prostatic fragments and vaginal fragments react to local hormonal stimuli (see Kullander 1956).

The slight "vicarious" oestrogen production in gonadectomized rats of this strain is remarkable as is the rarity of adrenocortical tumours. It is possible that these phenomena are interdependent. These R animals also have a low frequency of mammary tumours (Boot 1961). Of the 42 animals gonadectomized at 3 weeks and killed at 18–31 months only one was found to have a benign adrenocortical adenoma and another an adrenal medullary tumour. No other tumours were found in the experimental animals.

Abstract

The effect of gonadectomy on the adrenocortical production of sex hormones in male and female rats of the II strain was assessed from the growth of vaginal and prostatic grafts implanted in the right and left adrenals respectively.

In both sexes gonadectomy was followed first by a slight production of androgenic substances. This androgenic activity then decreased — in animals examined at 18 months it was very low — and afterwards increased — in animals studied at 31 months it was substantially higher particularly

Table 2 Degree of hormonal stimulation of prostate and vagina and of isografts of prostate and vagina implanted in adrenal cortex of the animals

Group	Sex	Prostate gland	Vagina	Graft of prostate in adrenal	Graft of vagina in adrenal
I	$+O_3$	(+)	—	(+)	—
IIa	$+O_3$	(+)	—	(+)	—
IIa	$+O_3$	(+)	—	(+)	—
IIb	$+O_3$	(+)	—	(+)	(muc)
IIb	$+O_3$	—	—	(+)	—
IIc	$+O_3$	—	(+)	(+)	—
IIIa	$+O_3$	+	—	+	—
IIIa	$+O_3$	++	—	++	(muc)
IIIb	$+O_3$	+	(+)	+	(muc)
IIIc	$+O_3$	—	—	+	—
IIIc	$+O_3$	—	—	(+)	—

exhibited secretory activity. The vaginal graft showed mucification. In the females of this group the prostatic graft and the vaginal graft was somewhat less markedly stimulated than in the males.

One of the female animals in this group had an adrenocortical neoplasm, a haemorrhagic cyst of the cortex with nodular cortical hyperplasia. Both the vagina *in situ* and the vaginal graft in the normal adrenal cortex in that animal showed a mucosa with proliferation and mucification of the cells. The prostatic graft in the adrenocortical tumour showed signs of secretion. These findings are most suggestive of production of androgen by the tumour.

In Group III c in which the grafts were implanted in association with

gonadectomy at 30 months, and in which the animals were killed one month later i.e. at the same age as the animals in Group III a and b, the prostatic gland *in situ* showed distended prostatic follicles bordered by fairly low epithelium with flat nuclei. The epithelium of the vaginal mucosa was low. The picture of the epithelium of the prostatic graft in the adrenal cortex in the male rats suggested moderate activity. The vaginal graft showed two layered epithelium. Similar, but less pronounced pictures, were seen in gonadectomized female hosts.

The results of the histological examinations altogether are given in Table 2.

Discussion

The fragments of the prostatic gland and vagina implanted in the adrenals appear to be able to respond to hormonal stimulation as long as the host lives. In one rat, in which by mistake only one of the gonads had been removed (Group II d) and which was killed 18 months after the implantation of the vaginal graft in the adrenal cortex the grafts showed the same cyclic phase as the vagina *in situ*.

At 2 months (Group I) Rats gonadectomized at 3 weeks roughly equally in males and females showed signs of slight androgen activity of the adrenals, as judged by the histological picture of the prostatic graft in the adrenal cortex. The oestrogenic activity on the other hand was extremely slight in both males and females. The production of androgen in young

gonadectomized animals has been observed by previous workers. In young rats the adrenal is said to have a special andromimetic effect (Burrill & Greene 1939). In animals that were allowed to live 18 months the androgenic as well as the oestrogenic activity was minimal (Group II a) after 31 months (Group III a) the androgen production was increased but only in the males.

Gonadectomy at 17 or 30 months (Groups II and III c) seems to produce about the same activity in the transplants in the adrenal cortex soon after the operation as does gonadectomy before puberty (3 weeks of age).

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The slight "vicarious" oestrogen production in gonadectomized rats of this strain is remarkable, as is the rarity of adrenocortical tumours. It is possible that these phenomena are interdependent. These R animals also have a low frequency of mammary tumours (Boot 1961). Of the 42 animals gonadectomized at 3 weeks and killed at 18–31 months only one was found to have a benign adrenocortical adenoma and another an adrenal medullary tumour. No other tumours were found in the experimental animals.

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The effect of gonadectomy on the adrenocortical production of sex hormones in male and female rats of the R strain was assessed from the growth of vaginal and prostatic grafts implanted in the right and left adrenals respectively.

In both sexes gonadectomy was followed first by a slight production of androgenic substances. This androgenic activity then decreased — in animals examined at 18 months it was very low — and afterwards increased — in animals studied at 31 months it was substantially higher particularly

in the males. Operative procedures (stress) seemed to increase the androgenic activity of the adrenals in these animals.

The production of androgenic substances did not appear to vary with the age of the animals at the time of gonadectomy (3 weeks, 17 months and 30 months).

The production of oestrogenic substances in gonadectomized rats of this strain proved low in both sexes, irrespective of age.

Only few adrenal tumours were seen in the animals studied.

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Studies on Aldosterone Production and Sodium Metabolism in Relation to Sympathetic Nervous Function in Man

By KERSTIN HALL and BERT HÖFELT¹

In the normal individual aldosterone production is closely related to sodium intake so that sodium restriction increases aldosterone production whereas sodium excess has the opposite effect (1-11). Patients with postural hypotension on the other hand have been reported to show no increase in aldosterone production following sodium restriction and to lose sodium under these conditions (14-18). These findings in patients with postural hypotension indicated a possible role of the autonomic nervous system in regulation of aldosterone production and sodium metabolism. In order to obtain further information concerning these questions we have studied the effect of sodium restriction on urinary excretion of aldosterone and sodium in two patients with postural hypotension and in addition in two normal subjects given guanethidine (Ismelin®) and pentolinium (Involvin®) in doses high enough to interfere with the function of the autonomic nervous system.

Materials and methods

Case 1 (411) a 64 year old man had a six year history of attacks of fainting in erect position, and marked fatigue and diminution after walking only a few meters which made him resort to squatting. In addition he had noted longstanding loss of sweating capacity in the left half of the body. Physical examination revealed no evidence of organic heart disease and blood pressure in supine position was 185/105 mm Hg. On standing for 30 seconds blood pressure decreased to 80/45 whereas pulse rate was unchanged. Laboratory tests showed hemoglobin 13.0 g per cent, normal serum electrolytes, urea 30 mg per cent and fasting blood sugar 87 mg per cent. The urine was free of sugar and protein, specific gravity after thirst 1.023. Thyroid function was normal with PBI of 2.6 microg per cent, normal adrenal function according to clinical and laboratory findings with daily urinary excretion of 17 ketosteroids of 9-10 mg and 17 ketogenic steroids of 13-16 mg per 24 hours and with proper increase after ACTH infusion. It had next urinary catecholamines were normal, noradrenaline ranging 7-11 microg and adrenaline 4-6 microg per 24 hours but there was no increase in urinary catecholamines in erect position. Electrocardiography at rest and x rays of skull and heart showed normal findings.

Case 2 (425) was a 63 year old man who had been operated for thyrotoxicosis in 1940 and duodenal ulcer in 1946. He was admitted

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because of attacks of fainting in erect position. He presented no signs of organic heart disease. In supine position blood pressure was 120/80 but decreased to 55/35 after 2 minutes in erect position with practically no increase in pulse rate. Hemoglobin was 12.8 g per cent serum electrolytes within normal range serum creatinine 0.9 mg per cent. There was no proteinuria and maximal urinary specific gravity 1.030. Fasting blood sugar fell within normal limits but there were trace amounts of sugar in the urine in a 24 hour collection and an intravenous glucose tolerance test showed decreased glucose disappearance. Thyroid function was normal with PBI 4.1 microg per cent radiiodine uptake over the thyroid 55 per cent after 24 hours and urinary excretion per 24 hours 35 per cent of administered dose. Clinical status and laboratory findings showed normal adrenal function with urinary excretion of 17 ketosteroids of 15 mg and 17 ketogenic steroids 20 mg per 24 hours. Urinary catecholamine excretion fell within normal limits (noradrenaline 10–12 microg and adrenaline 3–4 microg per 24 hours). No measurements were performed of catecholamine production in supine as compared to erect position. Electrocardiography at rest and x-rays of skull and heart showed normal findings.

Case 3 (A.S.) was a 21 year old man admitted because of lack of puberty. Final diagnosis based on surgical exploration was aplasia testis of unknown aetiology. There were no signs of other endocrine dysfunctions or any other organic disease. Thus physical examination of heart and circulation revealed normal findings. Laboratory investigations showed normal electrolytes, normal PBI (5.6 microg per cent), urinary excretion of 17 ketosteroids and 17 ketogenic steroids within normal range (4–9 mg and 9–17 mg per 24 hours respectively). Urinary total gonadotrophins were high (192 ME per 24 hours). Roentgenological examinations revealed normal sella turcica but delayed closure of the epiphyseal lines (corresponding to an age of 15–16 years).

Case 4 (H.A.) was a 19 year old man admitted because of hypogonadism. Physical

examination revealed no palpable testes well developed penis, scanty pubic and axillary hair and no beard. Urinary total gonadotrophins were high (96 ME per 24 hours) indicating primary testicular dysfunction. Laparotomy was not performed. Roentgenological examinations showed normal skull and normal skeletal development for the patient's age. According to clinical and laboratory investigations he had normal thyroid function (PBI 4.7 microg per cent) and normal adrenal function (urinary excretion of 17 ketosteroids 10–16 mg and 17 ketogenic steroids 11–19 mg per 24 hours, normal response following Metopirone® administration).

The studies were performed on the metabolic ward and the patients were allowed to be up and about during the day according to ability. Throughout the studies the patients were maintained on a constant diet supplying 13 mEq sodium per day. During control periods extra salt was added in an amount of 5 or 6 grams of sodium chloride per day (which corresponds to an ordinary salt intake). Following a control period sodium restriction was introduced by withdrawal of the extra salt. This was done in order to test the ability of the patients to increase aldosterone production and conserve sodium. To test the effect of adrenergic and ganglionic blockade on these functions Ismelin® and Ansolyse® were given to patients 3 and 4 both under conditions of extra salt and under sodium restriction. Ismelin® was given orally in doses of 20–30 mg per day which dosage induced pronounced postural reactions as judged from marked fall of both systolic and diastolic blood pressure on standing concomitantly there was an increase of pulse rate. Ansolyse® was given orally in increasing dosage from 10 mg to 40 mg four times a day which resulted in marked fall of systolic pressure on standing but with maintained diastolic pressure and with concomitant marked acceleration of pulse rate.

For the determination of sodium, potassium and aldosterone urine was collected in periods of 12 or 24 hours and stored in the cold but without preservative. In cases 3 and 4 urinary analyses also included creatinine

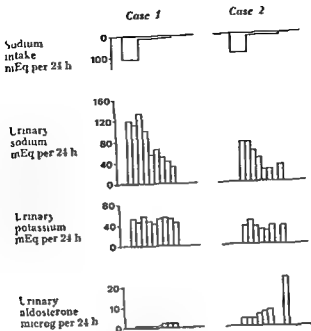


Fig 1 Effect of sodium restriction on urinary excretion of sodium potassium and aldosterone in two cases of postural hypotension

and catecholamines. Sodium and potassium were determined by flame photometry using an Eppendorf spectrophotometer (2) catecholamines fluorometrically according to Luler and Lishajko (11) for aldosterone the procedure of Neher and Weltstein (13) was used in cases 1 and 2 whereas an isotope dilution technique principally in accord with the method of Klimin and Peterson (10) was used in cases 3 and 4. For other laboratory investigations clinical routine procedures were used.

Results

Effect of sodium restriction on urinary sodium and aldosterone in postural hypotension

In the two cases studied renal sodium conservation was sufficient as judged from a maximal decrease of uri-

nary sodium to 32 and 24 mEq per 24 hours in case 1 and 2 respectively, on an intake of less than 13 mEq per day (fig 1). In both cases sodium restriction was followed by reduction of body weight probably indicating reduction of extracellular volume. There were no obvious changes in serum electrolytes.

Aldosterone excretion was subnormal (1–2 microg per 24 hours) in case 1 when maintained on an ordinary salt intake of 5 g per day and there was no increase in aldosterone following sodium restriction over five days. Case 2 on the other hand showed urinary aldosterone (4 microg per 24 hours) within the normal range on a salt intake of 5 g per day and fol-

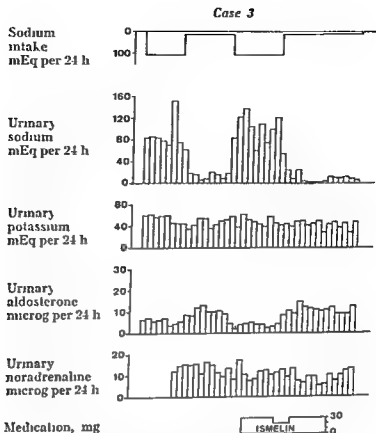


Fig 2 Effect of sodium restriction on urinary excretion of sodium potassium aldosterone and noradrenaline in a normal subject before and under treatment with guanethidine (Ismelin®)

lowing sodium restriction aldosterone increased to 24 microg per 24 hours. The procedure used for the determination of aldosterone in these two patients (see above) gives urinary excretion values of 2–8 microg per 24 hours in normal subjects on an ordinary diet, with about three-fivefold increase on sodium restriction.

Urinary excretion of sodium, catecholamines and aldosterone in healthy subjects during treatment with Ismelin® or Ansolyzen®

On a constant sodium intake of 6 g per day urinary sodium and aldoste-

rone remained unchanged following administration of Ismelin® and Ansolyzen®, respectively (fig 2 and 3). Furthermore during medication with these drugs sodium restriction induced a marked increase in urinary aldosterone and concomitant herewith, a pronounced fall in sodium excretion which was of the same order of magnitude as seen in normal, untreated subjects. There were no changes in serum sodium or potassium in connection with sodium restriction and/or treatment with Ismelin® or Ansolyzen®. Calculation of cumulative sodium balance during sodium restric-

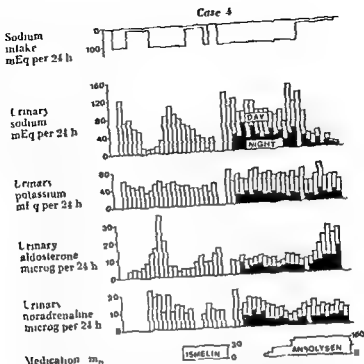


Fig 3 Effect of sodium restriction on urinary excretion of sodium potassium aldosterone and noradrenaline in a normal subject before medication and under treatment with guanethidine (Ismelin®) and pentolinium (Ansolysen®) in connection with Ansolysen® treatment urine was collected in 12 hour periods as indicated by black areas for the night and white areas for the day

tion showed the same result independent of whether Ismelin® was administered or not

During treatment with Ansolysen® urinary aldosterone was measured in periods of 12 hours and it was found to be significantly higher during the day as compared to the night (fig. 3)

During treatment with Ismelin® and Ansolysen® noradrenaline excretion tended to diminish but the decrease was not statistically significant. Ansolysen® did not influence the nor

mally occurring diurnal variation in noradrenaline excretion which was significantly higher during the day (fig. 3)

Comments

The results of the present studies in postural hypotension are similar to those reported by Wagner (18) and Birtter et al (1). Thus both patients failed to conserve sodium properly when put on sodium restriction. Furthermore in one of the patients uri

nary aldosterone was subnormal on ordinary sodium intake and did not increase on sodium restriction. In the second patient urinary aldosterone fell within the normal range when sodium intake was 5 g a day and increased markedly during sodium restriction. Sodium conservation was, however, still inefficient, which might indicate either that the increase in aldosterone production was not high enough for adequate sodium conservation or that this defect in sodium handling was not related to aldosterone production.

Since postural hypotension is considered to be due to lesion(s) within the sympathetic nervous system, earlier and present studies in this disease indicate that the sympathetic system can be engaged in regulation of aldosterone production and sodium metabolism. The lesions can be situated at various sites such as the autonomic brain centre, the efferent spinal cord, the sympathetic ganglions or the sympathetic nerves (3, 14, 15, 16, 17, 18) which no doubt explains why the postural reaction can be connected with various signs of autonomic dysfunction. A difference in location or degree of damage in the sympathetic nervous system was indicated also in our two patients and might have some bearing on the different responses in aldosterone production. Thus case 1, who showed no increase in aldosterone production following sodium restriction, also presented impaired sweat regulation, whereas this was not so in case 2, who responded with increased aldosterone.

Ismelin® and Ansolysen® are both potent inhibitors of sympathetic nervous function and can induce hemodynamic changes similar to those found in postural hypotension. Ismelin® exerts its effect by depleting tissue catecholamines and by peripheral blockade at postganglionic nerve endings (5). It produces hypotension in normal individuals by abolishing the normal increase in peripheral vascular resistance in upright position (6). Ansolysen® on the other hand, blocks the normal ganglionic reflex. In our studies treatment with Ismelin® and Ansolysen® resulted in some decrease in catecholamine excretion (although not statistically significant), and there was a marked effect on blood pressure. Thus, it is evident that the doses used in our studies were sufficiently high to interfere with the sympathetic function. Since treatment with Ismelin® and Ansolysen® did not interfere either with regulation of aldosterone production or sodium conservation, our studies support the view that peripheral efferent sympathetic pathways are not essential for aldosterone regulation in relation to electrolyte metabolism. This is further supported by the findings that Ansolysen® medication did not alter the normally occurring diurnal variation in aldosterone excretion (12).

The lack of any overt effect on sodium metabolism of the sympatholytic drugs used in our studies were unexpected in view of the results reported by others. For instance Giff et al (9) found that adrenergic blockade facilitates sodium excretion during a

pid sodium infusion in normal subjects. Furthermore Gill and Bartter (8) found that Ismelin[®] treatment during sodium restriction in normal individuals was followed by a cumulative loss of sodium and water.

Summary

Two patients with postural hypotension showed impairment of sodium conservation ability. In one of the patients aldosterone production was minimal on ordinary salt intake and did not increase on salt restriction. In the other aldosterone production fell within the normal range under ordinary salt intake and increased following sodium restriction.

When guanethidine and pentolinum were given to two normal subjects in an attempt to copy postural hypotension there was no evident abnormality either in sodium metabolism or aldosterone production.

Acknowledgements

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Attacks Simulating Pheochromocytoma in Patients with Angina Pectoris

By ALBERT SJOERDSMA

It is a pleasure for me to contribute in a small way to this volume honoring Jan Waldenström on his 60th birthday. I was privileged to come to know Professor Waldenström in our mutual interest in the carcinoid syndrome, which he was first to recognize and relate to excess serotonin production by the tumors. In 1959 I spent several months in his department and was exposed on a day to day basis to his great intellect and personality. Because of his almost instinctive ability to discover clinical and pathogenetic entities, I thought it would be appropriate to discuss briefly a clinical circumstance we have observed which may be fairly common but which has not been emphasized in the medical literature. This is the association of elevated blood pressure and chest pain in patients who have hypertensive episodes resembling those seen with pheochromocytoma but which may be attributed to coronary artery disease rather than excess catecholamines.

On a number of occasions in the past few years I have been asked to

perform chemical diagnostic tests for pheochromocytoma on urine from patients who had hypertensive attacks in association with chest pain. Even though the tests were negative, the physicians in charge of these cases were often reluctant to believe that the patient did not have pheochromocytoma. We have recently studied three such patients in our own institution, one had actually been subjected to a negative exploratory laparotomy. They were all men, aged 32, 59 and 62, who had repeated episodes of anterior chest pain with arm radiation accompanied by sweating, tachycardia and marked blood pressure elevations. Attacks lasted from a few minutes to an hour and occurred several times daily. The first two patients had attacks during a prodromal period preceding myocardial infarction after which pressor episodes ceased (Fig 1). The third patient had attacks for 5 years without development of infarct. Reported observations during attacks in this patient indicated that the onset of chest pain either preceded or coincided with

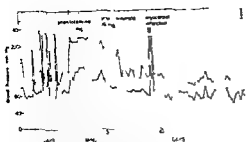


Fig 1 Hypertensive episodes occurring in association with chest pain in a 50 year old man. Effects of phentolamine and glyceryl trinitrate during one episode are shown. Note cessation of attacks after myocardial infarction.

the onset of increases in blood pressure and heart rate.

From these observations plus studies described in the literature showing the existence of pressor reflexes originating in various vascular beds we have entertained the hypothesis that the pressor attacks may be triggered

by reductions in coronary flow. Of course pain per se might also serve as a pressor stimulus. The disappearance of pressor attacks following myocardial infarction could be interpreted as due to destruction of areas provoking the pressor reflex. We also suspect that some cases of chronic hypertension may be due to coronary artery disease. Certainly disappearance of chronic hypertension after myocardial infarction has been widely noted. Horwitz and I have discussed this and other associations between blood pressure elevation and angina pectoris in more detail elsewhere (1).

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The fate of a Morbus Cushing Case

24 years' follow up

By HELGE B WULFF

In the history of the pathophysiology of the adrenal glands and the treatment of the adrenal diseases, the occasional isolated observation has played a dominant and stimulating rôle. Ever since *Cushing's* primary findings, *Philip Hench's* (1) observation of improvement of rheumatic patients during pregnancy and in certain cases of jaundice and to *Hendall's* (2) and *Hench's* brilliant collaboration on the basis of occasional bizarre findings parallel with the *Reichstein* group all the endeavours made have lead to the final triumph — the presentation of cortisone. This outstanding achievement was already begun in 1929 by *Hartmann* and *Swingle & Pfiffner*.

The occasional isolated observation in the laboratory or at the bedside has by no means lost its importance. The new age, that in the near future will have the electronic computer as an ensignia, is more than ever dependent on just these isolated observations for its further development. However ob-

servations alone are not enough. The ability to combine the observations with those things occurring in the world, often in completely different fields, is also of the greatest necessity.

But also the occasional isolated recording of a course of a disease, a history over a long period of time, often makes analyses and evaluations possible which are of importance for future action.

When the cortisone was introduced into clinical use in 1950 new discussions were taken up as to the possibilities of bilateral adrenalectomies in those cases of Morbus Cushing, in which the disease was supposed to be caused by hyperplasia of the adrenal cortex. According to our present knowledge this is probable in approximately 60 % of these cases. This method of radical therapy with the help of cortisone was soon adopted by surgeons and endocrinologists all over the world and careful recordings were made of the pre- and postoperative conditions together with the immediate risks and

possibilities. The whole panorama of the disease, with special consideration to late results of many years' duration, however is still of importance not least in regard to possible complications.

A case of Morbus Cushing followed up for more than 24 years of which more than 7 years after bilateral adrenalectomy shows a course and characteristics which might be of some general interest.

History. A previously completely healthy young nurse — L.S. born 1910 — without hereditary characteristics was in 1942 (at about 32 years of age) admitted to a University Clinic in Sweden for amenorrhea of a few months duration the cause of which could not be explained. After careful investigations — including hormonal analyses — her condition was interpreted as hypophyseal insufficiency but at the same time she showed a typical Cushing appearance with moon face and striae and so on. Blood pressure 180/120 serum calcium 10.7 mg% serum phosphorus 3.2 mg% Roentgen examination of the skeleton including the skull revealed normal sella and no osteoporosis was present. Intravenous urography was normal. She was given a certain stimulating and roborant treatment. The amenorrhea disappeared in 1946 and 1949 she again had short periods of amenorrhea and transplantsations of pituitary gland were performed. Normal menstruations until the menopause in 1957.

During the years 1942—1957 the patient held a demanding position as head nurse and matron at a hospital. She however showed only increasing Cushing disease and increased 10 kg in weight. Blood pressure 180/110. At the age of 45 (1955) she had two periods of mental depression. During the same year she also suffered an ulceration of her lower leg with very slow wound healing which caused plastic surgery.

In connection with the menopause in 1957 (4 years of age) increasing abdominal obe-

sity purple complexion moon face buffalo back vascular brilliency pigmentation of the lower extremities which had become very gracile hyperglycaemia (0.20 blood sugar on fasting) total eosinopenia, increased 17 k G S. ACTH test positive for Cushing increased 17 k S. Roentgen examinations of the kidneys indicated a certain hypertrophy of the right adrenal gland. All findings argued for a Morbus Cushing caused by hyperplasia of the adrenal cortex.

In January 1958 (at the age of 48) right sided adrenalectomy was performed without complications (weight 8 gm) microscopic diagnosis hyperplasia of the cortex.

No marked improvement was noted after the operation. In November 1958 she suffered an obscure cerebral attack with paresis of the left arm and leg which however subsided after some time. Blood pressure 190/110.

Considering the fact that the removal of the right adrenal did not seem to have brought about an improvement of her condition a left sided adrenalectomy was performed in February 1959 (prep 7 gm). No complications occurred during the operation. A slight pneumothorax was corrected. The Cushing picture began slowly to become normalized. The blood pressure went down to 145/105 on an amount of 40 mg cortisone/day. Half a year later the patient returned to work and accepted a demanding position at a hospital. She was however under a heavy mental pressure with depressions and mental weakness. The adequate treatment with cortisone caused marked complications and she exhibited a picture pendulating between Cushing's disease and that of Addison.

First in 1960 a definite improvement occurred and the blood pressure became stabilized at 170/110. During the years 1961—1964 she worked part time with interruptions for mental depressions on a few occasions. In 1964 the patient suffered a slight trauma of the right lower leg causing a slow healing ulceration which required a long treatment. During that time also symptoms from the abdomen with repeated spells of pain of unknown origin. Thorough investigations gave a negative result.

In May 1963 the patient was operated upon on the suspicion of acute appendicitis. The appendix was healthy. In the postoperative course a certain infiltration was noted in the scar.

The pains continued — at times unbearable — and explorative laparotomy was performed in June 1963. A tumour like infiltrate was found in the scar (microscopic diagnosis: granulation mass with necrosis of fat tissue). A band over a loop of ileum of the same appearance as the scar (microscopic diagnosis: necrosis of fat tissue) was found on entering the abdomen. The band was removed. The patient then recovered and the abdominal pains disappeared. The band and the infiltration of the abdominal wall were interpreted as having been caused by an extended cortisone treatment.

The external picture of Morbus Cushing has now almost disappeared. Blood pressure 130/90. Roentgen examination of the skeleton showed a moderate osteoporosis. From time to time she catches colds but in general her condition is satisfactory.

In a case of Morbus Cushing diagnosed in 1942 (at 32 years of age) the dominant features for 15 years were Morbus Cushing habitus, a blood pressure of 180/110 mm/Hg, a certain irregular menstrual cycle, slow healing ulcerations of the lower extremities and periods of mental depression. The patient, however, had full working capacity for almost the whole time. In 1958 a right sided adrenalectomy was performed without improvement of the patient. In 1959 the left adrenal was removed. Thereafter the patient experienced rather disturbing effects from the substitution therapy and for 1—2 years she was mentally very much invalidated but there was a clear improvement of the Cushing picture. Seven years after the first adrena-

lectomy she showed only slight symptoms of her original disease.

The substitution therapy with cortisone has for the past eight years caused the patient trouble in the form of frequent respiratory infections, periods of mental depression, slow healing wounds after slight traumata and a period of abdominal pains caused by the occurrence of a band over a loop of ileum and necrosis of the abdominal wall which may be due to the administration of cortisone over a long period of time.

Due to the patient's great ambition, she has been able to carry on her work for almost 23 out of the 24 years that have elapsed since the onset of the disease.

Conclusions

In cases of Morbus Cushing with certain or suspected hyperplasia of the cortex bilateral total adrenalectomy should be performed. Unilateral procedure is advisable only in cases where a localized adenoma is demonstrable. The administration of the substitution therapy requires very thorough control in regard to mental variations, blood pressure, nosocomial infections, respiratory conditions, etc. A great many abnormal conditions not anticipated can develop in the digestive tract or elsewhere in the form of inflammatory lesions, necrosis and 'tumors' when administration of cortisone takes place over a long period of time.

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tinuous control by specialists in hospitals with laboratories and other necessary equipment at their disposal. Without these possibilities surgical therapy is nowadays contraindicated.

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The So-Called Growth Hormone of the Anterior Pituitary

By ROSE LUTZ

More than 40 years ago Evans and Long demonstrated for the first time that extracts prepared from the anterior pituitary had growth promoting activity. The substance responsible for this action was of course called growth hormone. It has been purified and shown to be a polypeptide. By the middle of the 1950's we had obtained a fair amount of knowledge about the widespread metabolic action of growth hormone, and it had been suggested that it might play a role in the homeostasis of the body's internal environment. These experiments were in animals. Our information about the action of growth hormone in man was surprisingly poor. The reason for this was twofold. Growth hormone from animal sources had been tested in man and though minor metabolic changes had been obtained with such preparations the results were, on the whole, disappointing. Furthermore, accurate and easily accessible method for determination of growth hormone in body fluids was not available. These two obstacles for studies on the physiology and pathology of

growth hormone in man were eliminated during the late 1950's. In 1956 Li and Papkoff (7) reported on the extraction and purification of growth hormone from human pituitaries (human growth hormone HGH), and showed that it differed in many of its characteristics from bovine growth hormone. This preparation was highly active in man and it was established that growth hormone to some extent was species specific. This was the reason for the earlier failures to obtain consistent effects with animal growth hormones in man. Furthermore, the introduction by Berson and Yalow (15) of the radioimmunoassay principle for quantitative determination of insulin in blood also opened the road for quantitative determination of other hormones of polypeptide nature in blood. Today several research groups throughout the world are taking advantage of HGH determinations for clarification of the biological functions of the hormone. These studies have shown that in most instances the effects of HGH are in accord with what is known from

animal work. In the following presentation these effects will be divided into two groups: those related to growth and those more intimately connected with carbohydrate and lipid metabolism.

Human growth hormone and growth

The findings so far have clearly demonstrated the paramount effect of HGH on growth or, in a wider sense on protein synthesis (1-4). Indirect signs of anabolism have included a lowering of blood urea and a positive balance of nitrogen and potassium plus a considerable retention of sodium with expansion of the extracellular space. All of these findings are consistent with the laying down of tissue. Other more direct signs of the growth promoting action of HGH include the therapeutic effect of HGH when administered to children with dwarfism due to deficiency in the production of HGH by the hypophysis and the resistance to such treatment in instances where the body has produced antibodies to previously administered HGH. The increased concentration of HGH in plasma from acromegalic patients and the disappearance of the hormone in blood after hypophysectomy in such patients similarly supports the growth promoting action of HGH.

The mechanism of action of growth hormone in protein synthesis is only known in its rough outlines. It may now be considered proven that growth hormone stimulates the initial step in protein synthesis that is the transfer of amino acids from the

extracellular to intracellular compartments (5). Much less is known about the significance of growth hormone on protein synthesis after the entry of amino acids into the cells. The evidence available at present — though in many respects contradictory — generally favors the concept of a direct action of growth hormone on one or several processes that regulate protein synthesis in the cell.

Human growth hormone and the metabolism of carbohydrate and lipids (2, 3, 5, 13)

A great deal of information has been gained during the last few years regarding the action of animal growth hormone on carbohydrate and lipid metabolism. On the whole these studies have added a new dimension to the earlier picture of the significance of growth hormone for metabolic processes. Growth hormone administration leads to a reduction of lipid synthesis and induces a precipitous rise in plasma free fatty acid concentration due to mobilization of free fatty acid from adipose tissue (11). This important lipid mobilizing action is not a direct one on adipose tissue and the mechanisms involved remain obscure. The significance of growth hormone for the further metabolism of lipids, the fatty acid oxidation is not clearly understood. Indirect evidence for such a stimulatory action has been suggested by the decrease in fat in the carcass of the animal given such hormone preparations, the increased ketogenesis and the depression of the respiratory quotient. The important role of growth hor-

more in the regulation of carbohydrate metabolism has been treated extensively and comprehensively in numerous reviews in recent years (5). The anti-insulin and diabetogenic actions of the hormone were clearly demonstrated 20 to 30 years ago (16). Recently, Randle and his colleagues (13) have proposed a challenging hypothesis for the combined action of growth hormone on lipid and carbohydrate metabolism. In its essence the hypothesis favors the idea that the anti-insulin and diabetogenic effects of the hormone are secondary to its lipolytic effect. Growth hormone promotes the mobilization of fatty acids and ketogenesis is accelerated. Since the oxidation of these substrates by muscle seems to be preferential to that of glucose the uptake of the glucose is inhibited.

The effect of HGH on lipid and car

bohydrate metabolism in man has been closely studied by our group and several others. HGH has been shown to exercise a 'diabetogenic' action in humans. A diminished glucose tolerance can be produced in healthy subjects by administering HGH. Fasting hypoglycemia, glycosuria, and sometimes ketonemia can be induced in hypophysectomized non-diabetic patients by giving them HGH. Thus a state of 'idiopathic diabetes' comparable to that found in animals is seen in hypophysectomized juvenile diabetics. Very small doses of HGH produce a dramatic deterioration of the diabetic state in these patients. Metabolic acidosis and insulin resistance are characteristic

These effects of HGH are accompanied by an increased release of free fatty acids and, as shown by indirect means, by a relatively decreased peripheral uptake of glucose (3). Such a diminished peripheral utilization of glucose by muscle tissue following HGH has since been demonstrated by Rabinowitz and Zierler (12) in their studies on the human forearm. Another aspect of the physiological role of HGH is currently being studied and that is its significance for blood glucose homeostasis. HGH in plasma is increased following insulin hypoglycemia (14). Plasma adrenaline, cortisol and glucagon levels are also elevated. In these tests without exception there has been a decrease in blood sugar to values which are never encountered in normal life and, therefore they do not necessarily give us information about the hormone's physiological significance in this respect. We may assume that the blood sugar under normal conditions, does not fall more than 10—15 mg per 100 ml. The response of HGH to such a minor change in blood sugar is demonstrated in Fig. 1 (taken from an unpublished article by Luit. Madison and Cervi). In this young healthy woman a blood sugar decrease of 15 mg per 100 ml (obtained by the infusion of 0.01 IU insulin per kg body weight for one hour) was accompanied by a remarkable HGH response while the excretion of adrenaline was unchanged. This clearly demonstrates the profound effect of HGH on blood sugar homeostasis.

Effect of prolonged small decrease
in blood glucose on HGH in plasma

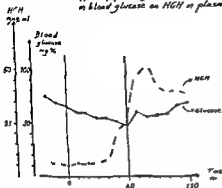


Fig 1 Effect of prolonged small decrease
in blood glucose on plasma HGH in a healthy subject

The relationship between the action of growth hormone on protein synthesis and on lipid and carbohydrate metabolism. If such a relationship between the protein synthetic and adipokinetic insulin antagonistic properties of the hormone exists it has been distinctly obscure.

The two groups of actions may reside in one and the same molecule (10). The hormone would then be protein sparing both by its actual effect on protein synthesis from endogenous amino acids and by its antiketotic action. Fatty acid mobilization would be a part of the latter function. Fatty acids spare glucose but this is equivalent to protein sparing during fasting since the glucose spared would otherwise have to be provided from amino acids. The theory is supported by the numerous findings that growth hormone is secreted in increased amounts when the protein stores of the body are threatened during fasting, hypoglycemia, muscular exercise etc.

Effect of HGH on energy homeostasis
and growth

Theory II

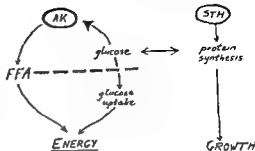


Fig 2 The Levine Luft two factor theory
on the effect of HGH on energy metabolism
and growth

On the other hand it is rather difficult to see the biological advantage in having the adipokinetic and protein synthetic activities of the hormone reside in the same molecule especially when the production of a "growth hormone" is influenced so markedly by every days life and in particular by even minor changes in blood sugar. For this purpose Levine and Luft (6) have introduced a theory (Fig. 2) which suggests that the so called growth hormone fraction of the anterior pituitary is composed of two hormonal entities the somatotrophin proper and the diabetogenic or adipokinetic component. The somatotrophin would promote protein synthesis (together with insulin) cause orderly proliferation of epiphyseal cartilage and possibly lead to increased liberation of insulin from the pancreas. The total effect of somatotrophin would consist of growth in body length and enhanced protein synthesis. The adipokinetic compo-

ment would stimulate the liberation of fatty acids from adipose tissue, thus shifting cellular metabolism to the utilization of fat whenever there develops a relative scarcity of carbohydrate. In this way, carbohydrate would be conserved for the use of the central nervous system which cannot readily use fat derivatives.

Future studies will show which of the one factor or two factor theories is the right one. Several reports have demonstrated the actions outlined above for the Levine Luit hypothesis. For instance, it has been shown that growth hormone preparations can and do release insulin from the β -cells in the intact dog and in man, which may explain the hypoglycemia and fall in the free fatty acid level of the blood which is seen soon after the administration of HGH.

Prolonged administration of HGH gives rise to a situation contradictory to the one just described, one in which high insulin liberation is accompanied by insulin resistance and decreased glucose tolerance. It is noteworthy that periods of rapid body growth are not accompanied by impaired carbohydrate and fat metabolism, and insulin resistance does not develop. Conversely, during fasting when carbohydrate utilization diminishes and the level of free fatty acids is increased there is no evidence of insulin release. Such findings are difficult to explain on the basis of a one hormone theory.

This new concept for the explanation of the various actions of the so

called growth hormone seems biologically advantageous. It suggests a variety of experimental approaches for testing its validity. The purification and fractionation of growth hormone preparations have to be further developed, and the hitherto sparse findings of fractions possessing one but not the other of the two activities have to be carefully tested.

Whether such studies may show, there can be no doubt that the pituitary growth hormone has progressed from its role as stimulator of bodily growth and under special conditions, as a "diabetogenic" agent to a key position in the regulation of body metabolism and energy homeostasis.

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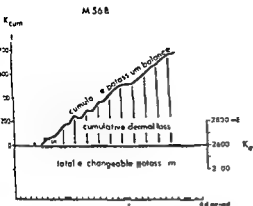


Fig 1 Cumulative potassium balance and total exchangeable potassium (K_{ex}) in a 92 day metabolic study. The increasing discrepancy is interpreted as caused by dermal losses of potassium.

due to dermal losses of potassium. From such calculations dermal losses of potassium were estimated to 2.2–9.9 ml q per day (mean value 6.5 mLq per day) in 12 different studies.

In Fig. 2 another example is presented where the error introduced by dermal losses can be compared with some other systematic errors occurring in balance studies due to "invisible returns" (Isaksson & Sjögren '2) and blood withdrawn. The cumulative cutaneous losses (500 mLq of potassium) were large in relation to the true changes in potassium balance during the study (about 100 ml q).

Dermal losses of sodium

The dermal losses of sodium may be determined in a similar manner (Fig. 1). In 7 studies performed under non-sweating conditions the losses were estimated to 2–14.0 ml q per day. Total exchangeable sodium amounts

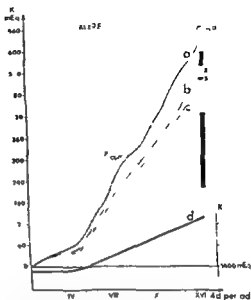


Fig 2 Systematic errors in a 64 day potassium balance study where the true changes are measured by the increase in exchangeable potassium (curve d).

Curve a = uncorrected cumulative potassium balance

b = cumulative potassium balance corrected for "invisible returns" (on trays, pots and pans)

c = cumulative potassium balance corrected for "invisible returns" and blood withdrawn

The difference between curve c and curve d represents dermal losses of potassium

to only 70 per cent of total body sodium while the corresponding figure for potassium is 93. A prerequisite for our calculations is therefore that the non-exchangeable fraction of bone sodium is not altered during the study.

Dermal losses of calcium

The dermal losses of calcium have been estimated from the determinations of cutaneous losses of potassium

Dermal Losses of Nutrients and their Significance for Human Metabolic Balance Studies

By B ISAKSSON, B LINDHOLM and B SJÖGREN

There are a great number of publications concerning the concentration of different nutrients in sweat (for references see Robinson & Robinson, 1) while reports on the daily total dermal loss of these components are considerably less frequent (Isaksson & Sjögren, 2). With the exception for sodium, these losses have been neglected in practically all human metabolic balance studies published so far. The danger of misinterpretation of balance data created through this negligence was pointed out early (Mitchell & Hamilton, 3), however and recently, repeated warnings have appeared against this danger in articles by Walker (4) by Mitchell & Edman (5) and by Consolazio and co workers (6, 7, 8).

At our Metabolic Unit we have worked out methods for the determination of the daily total dermal losses of potassium, sodium, calcium and nitrogen, which will be summarized here. A few examples will show how the negligence of such losses may cause serious misinterpretation in orthodox balance studies which are

based on analyses of food, urine and faeces only.

Dermal losses of potassium

Recently, we accounted for a method for the indirect determination of the dermal losses of potassium (Isaksson & Sjögren, 9). The principle of the method is demonstrated in Fig. 1. During an orthodox potassium balance study (Methods, see Isaksson & Sjögren, 10) total exchangeable potassium is determined repeatedly by an isotope dilution technique (Corsari *et al.* 11). This gives us two different expressions for the changes in total body potassium, K_{cum} and ΔK_{ex} , where K_{cum} represents the cumulative potassium balance and ΔK_{ex} the changes in total exchangeable potassium. They should obviously agree but Fig. 1, based on a balance study of 112 days, shows that this is not the case. In other long term balance studies we have regularly found similar discrepancies with respect to potassium. As other systematic errors have been considered it appears reasonable to explain the discrepancy

discrepancy between the cumulative nitrogen balance and the Δh_{ex} (Fig 4). Calculated in this way the dermal losses of nitrogen amounted to 0.2—1.6 g per day (mean 0.9 g) in 10 cases.

Discussion

Our indirect determinations of the dermal losses of nitrogen have given results which are close to the figures for integumental replacement reported by Mitchell (13). In adult persons assumed to be in nitrogen balance he regularly found a positive balance of this element which was supposed to correspond to "growth of the skin and the epidermal structures". Direct measurements of nitrogen losses with sweat however give less conspicuous results presumably because desquamated epithelium is included only to a minor degree. Using sweat analyses and water balance Mitchell & Hamilton (3) found figures for daily losses of nitrogen of 0.36 g per day only.

Determinations of cutaneous losses of potassium and sodium from analyses of underwear plus skin washings have given results similar to our indirect estimations when sweat was collected for 24 hours (Freyberg & Grant 14; Kaufmann *et al.* 15; Arn & Reimer 16). The figures of Dahl *et al.* (17) and of Gellman & Lutwak (18) on the other hand are considerably lower. It is possible however that during these conditions electrolytes are reabsorbed from the skin and clothes. Some support for this assumption can be found in Dahl's data showing the same amount of electrolytes in the clothes

worn for seven consecutive days as in those worn for only three days.

It is of great importance to consider dermal losses in metabolic balance studies. The error introduced from negligence of these losses often accumulate to figures which are large in relation to the true changes as exemplified in Fig 1—3. The same holds true for calcium and in a few studies the apparent positive calcium balance were even changed into definite negative ones after correction for cutaneous losses of calcium (Isaksson & Sjögren to be published).

Fig 4 illustrates how a nitrogen balance study can be misinterpreted from the data of an orthodox balance. It is taken from a study of the effect of anabolic steroids on the nitrogen balance in an asthmatic subject treated with cortisone. The apparent nitrogen balance was positive throughout the study and accumulated to 120 g in 92 days.

This should correspond to a lean tissue synthesis of 3.6 kg. The observed weight increase was 3.2 kg. Taking the h_{ex} figures into account however we have concluded that the increase in weight was caused by fat accumulation and/or water retention. Reduction in caloric supply stopped further increase in weight but failed to influence the apparent positive nitrogen balance.

Summary

Methods for indirect determinations of dermal losses of potassium, sodium, calcium and nitrogen during long

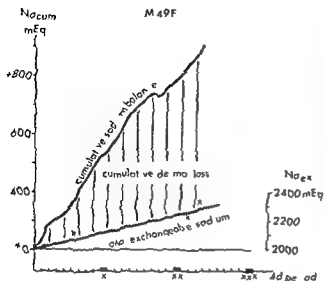


Fig 3 Cumulative sodium balance and total exchangeable sodium (x) in a 92 day metabolic balance study. The increasing discrepancy is interpreted as caused by dermal losses of sodium

already described. If the relation of Ca/k in whole body sweat is known the dermal losses of calcium (Ca_{derm}) can be calculated from the expression

$$Ca_{derm} = k_{derm} \times (Ca) / (k)_s$$

where k_{derm} stands for the daily dermal loss of potassium (Ca_s) and $(k)_s$

is the concentration of calcium and potassium in sweat. Data from our laboratory have shown that in the same person this ratio does not vary considerably from day to day nor during the day. It has also been found that the ratio Ca/k determined on sweat samples from the arms are representative for whole body sweat (Isaksson & Sjogren to be published). The dermal losses of calcium estimated in 7 cases ranged 19–300 mg/day (mean 111 mg/day).

Dermal losses of nitrogen

Repeated determinations of k_{ex} during a nitrogen balance study make it possible to estimate the dermal losses of nitrogen. Total body potassium can be considered as a parameter of the cell mass. Changes in total exchangeable potassium thus reflect changes in total body nitrogen. Under the assumption of a constant k/k ratio in the body (3/1 according to Fikinton & Drowski 12) the dermal losses of nitrogen can be calculated from the

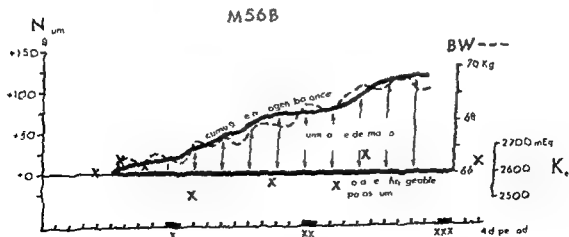


Fig 4 Cumulative nitrogen balance (—) body weight (---) and total exchangeable potassium (x) in the same study as in Fig 1. For explanations see text

MISCELLANEOUS SUBJECTS

term metabolic balance studies are described

The daily dermal losses amount to 2–10 mEq of potassium, 3–14 mEq of sodium, 20–300 mg of calcium, and 0.2–1.6 g of nitrogen respectively.

A few examples are given to illustrate the significance of these losses for the interpretation of balance studies.

Acknowledgement

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MISCELLANEOUS SUBJECTS

Cerebral vascular disease

By GEORGE PICKERING

Regius Professor of Medicine University of Oxford

With the decline in the death rate from infectious diseases people are living longer and dying mainly from heart disease cancer and strokes Of these by far the least well understood is the latter

One of the main difficulties in the investigation of cerebral vascular disease is to display vessels in their continuity The standard method of slicing brains leaves large segments uninspected and it is not easy to be sure which vessels lead to which In addition a recent haemorrhage produces extensive disruption of the brain and an old lesion is difficult to identify with certainty

Three kinds of vascular disturbance may produce the sudden interruption of cerebral function which we call a stroke Embolism or thrombosis may interrupt the flow of blood haemorrhage may produce a rapidly expanding space occupying mass Embolism can usually be diagnosed only if a cause is found e.g. rheumatic heart disease with mitral stenosis or auricular fibrillation or both auricular fibrillation by itself myocardial infarction and occasionally a paradoxical

embolus or a metastasis To distinguish clinically between thrombosis and haemorrhage is extremely difficult I am now convinced that I cannot do it without an angiogram

The most important advance in our understanding in this field has been the recent introduction by Ross Russell (1963) of a satisfactory method of displaying the cerebral vessels in their entirety His method had previously been developed by Mitchell and Schwartz for displaying the coronary vessels Briefly the carotid arteries were cannulated and the basilar ligated The brain was then suspended in water at 40° C for ten minutes and the arterial tree irrigated with 10% formol saline and then barium sulphate in gelatine Subsequently the hemispheres were cut coronally with a domestic meat slicer and the slices cleared by Spartholz's method Using this method Ross Russell examined the brains of 24 subjects freshly coming to necropsy in Boston City Hospital the diastolic pressure was over 110 mm Hg in 16 below in 38 subjects Small aneurysms were found in all but one of the hyperten

sive group and in 10 of those with lower pressures. With one exception, patients with more than 10 aneurysms all had hypertension. No aneurysms were found in patients under 50 years. These aneurysms were situated on small arteries 100–300 microns in diameter, particularly on lateral branches of the striate arteries or on penetrating vessels from the cortex. The muscular tissue of the parent vessel terminated abruptly at the point of origin of the aneurysm and the remnants of elastica could be seen to extend for a short distance into the aneurysm before disappearing. The wall of the aneurysm was composed of connective tissue only, with an inner hyaline layer derived from intima fusing with an outer collagenous layer continuous with the adventitia of the parent vessel. Frequently the aneurysms showed evidence of leaking and thrombosis. Ross Russell's observations suggest that such aneurysms form continuously in patients with elevated pressures as they grow older. They can produce small haemorrhages and small thromboses with local softening which would resemble the miliary infarcts described by Rosenberg (1940). When the thromboses extend proximally into larger arteries larger areas of softening may result. Finally there may be massive rupture with massive intracerebral haemorrhage. It would seem likely that these intracerebral aneurysms are the missing link between raised arterial pressure and cerebral vascular disease.

Another development of some importance has been the discovery of the

role of embolism. There now seems little doubt that the premonitory symptoms of carotid artery thrombosis are due to the detachment of platelet emboli from the nodule in the carotid artery (Gunning *et al.*, 1964). These emboli may be so friable that they pass through the retinal and cerebral circulations, producing only a temporary interruption of the blood supply, or they may stick permanently with more lasting consequences. It is quite possible that occlusions of arteries of the size of the middle cerebral, posterior cerebral or cerebellar arteries are mostly embolic from mural thrombi dislodged from nodules on the carotid or vertebral arteries.

Thus it would seem that cerebral vascular disease is comprised of at least three separate disease processes.

- 1 The berry aneurysms on the arteries of the Circle of Willis and the main branches arising from it. These may be congenital or mycotic; they commonly rupture to give the familiar syndrome of subarachnoid haemorrhage.

- 2 Nodular arteriosclerosis (Councilman, 1891; Pickering 1964) of the carotid and vertebral arteries. This is a generalised vascular disease whose most familiar manifestation is the syndrome of myocardial infarction. Affecting the carotid and vertebral arteries it may produce ischaemia of the hemispheres or the brain stem possibly mostly by embolism. This disease is loosely correlated with arterial pressure and is especially frequent in males.

3 Microaneurysms of the perforating arteries of the brain. These may give rise to cerebral haemorrhage or minute infarctions. This disease seems to be correlated particularly with age and arterial pressure. It is probably responsible for the high correlation between elevated arterial pressure and death from cerebrovascular disease.

One of the tragedies of our time is a neglect of the proper use of words. A technical term should describe as far as possible a fact or an idea accurately and it should have only one meaning. Atherosclerosis means literally "hardening through the agency of porridge"; cerebral atherosclerosis means "hardening of the brain through the agency of porridge". It would be a little difficult for a scholar unused to the misuse of technical jargon to discover that what was refer-

red to was vascular disease of the brain. But the chief crime of this term is that it confuses two diseases which are utterly different in location, form and causation. We shall never unravel the secrets of nature — which is supposedly the purpose of medical research — unless we learn to distinguish unlike phenomena one from another. If this article has done this, it will have served its purpose.

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Waldenstrom's Chronic Active Hepatitis

By SHEILA SHERLOCK

In 1950, Jan Waldenstrom spoke to a meeting on Digestion and Metabolic Diseases at Bad Kissingen in the Black Forest. The title of the paper was "Leber, Blutproteine und Nahrungs-eiweiss". In this lecture he referred to a particularly interesting group of young people, predominantly girls during or shortly after puberty, suffering from a form of chronic liver disease. The general features were moderate jaundice with intermittent bilirubinuria and in the later stages, with ascites and oesophageal varices. In almost all cases there were spider naevi and some Amenorrhoea which was anovulatory, was typical. Erythrocyte sedimentation was increased. Flocculation tests were positive. Serum electrophoresis revealed that the albumin was probably normal but gamma globulin was greatly increased. The alpha₂ and beta globulins were maintained. This then is the first description of the condition which has since attracted much attention and been described under many names. The obscurity of the journal in which this original paper was published has

given countless authors and editors difficulty in checking and the reference to it has been omitted in many publications. The paper on the same subject by Kunkel and the Rockefeller group appeared in 1951 and the other account of the same condition was by Bearn and co workers (1956) under the title "Chronic liver disease in young women". Willock and Isselbacher (1961) called it "Chronic liver disease in young people". A rather similar condition in women around the age of the menopause was reported by Cattan and co workers (1957) under the title "Cirrhoses dysprotéiniques d'origine inconnue chez la femme". The association with a lupus erythematosus like syndrome and with L.E. cells in the blood was noted by Joske and King (1955) and Krook (1961). Mackay and his colleagues (1956) from Australia coined the term "lupoid hepatitis". The prominent cellular infiltrate led Good (1956) to use the term "plasma cell hepatitis". Read and co workers (1963) used the term "Active juvenile cirrhosis" although they recalled that the condition was

not always a cirrhosis in the earlier stages and did not always affect young people

Present Patients

The present material comprises 561 patients with various types of cirrhosis of the liver seen by the author at the Royal Free Hospital London between October 1959 and October, 1965. The definition of cirrhosis is widespread hepatic fibrosis with nodule formation. In most instances the diagnosis has been made on histological grounds. They have been divided into five groups. Cirrhosis of the alcoholic (134 patients), active chronic hepatitis (139 patients), primary biliary cirrhosis (75 patients), haemochromatosis (29 patients) and Wilson's disease (13 patients). The largest group called cryptogenic (171 patients) had no significant aetiological factor. Patients with biliary secondary to extrahepatic biliary tract obstruction have been excluded and also the few cases of genuine cardiac cirrhosis. These 561 cases represent a spectrum of chronic liver disease seen in a hospital which has a special interest in the condition. It in no way reflects the incidence of liver disease in Great Britain as a whole. In fact Britain has one of the lowest incidence rates of cirrhosis in the world. This paper is concerned with the active chronic hepatitis group its diagnosis, course and treatment and its relation to other forms of cirrhosis seen in Britain.

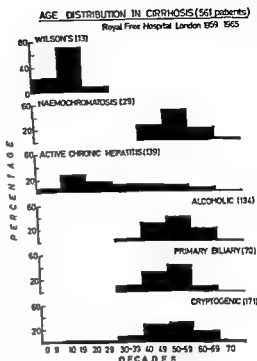


Fig 1 The age distribution of cirrhosis in the Royal Free Hospital London. Note that 48% of the 139 patients with active chronic hepatitis were between 10 and 59 years old.

Criteria for Diagnosis

Clinical The condition is predominantly one of young people especially women presenting with persistent hepatocellular jaundice. In the present series 48% were between 10 and 29 years old (fig 1). This contrasts strikingly with the alcoholic, cryptogenic, primary biliary cirrhosis and haemochromatotic groups who have a peak incidence between 40 and 59. Nevertheless the condition can occur in childhood, the youngest patient being 6 years old and also on into later life, the oldest being 75 years. The

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Fig 3 Active chronic hepatitis There is a profound predominantly mononuclear cellular infiltrate spreading in from the portal zones Liver cells are isolated into groups and vary in size Stained H & E $\times 120$

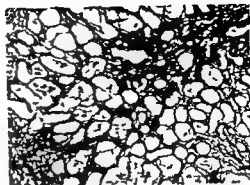


Fig 4 The liver cells are divided into groups by dense reticulin which is in parts aggregated into bands where liver cells have disappeared The zonal architecture is completely lost Stained Reticulin $\times 120$

of liver cells and eventually a post necrotic cirrhosis. The fibrosis varies from portal zone enlargement through isolation of groups of liver cells in a network of dense connective tissue to frank cirrhosis with easily identifiable nodule formation. The junction between liver cells and fibrous tissue is irregular the connective tissue actively encroaching on the parenchyma. The early stages are those of a chronic hepatitis and later the picture is of a true postnecrotic (mainly macronodular) cirrhosis. When needle biopsy material only is available because of the small sample size it may be extremely difficult to be sure whether or not a true cirrhosis is present. Liver cell damage is usually profound with vacuolation of the peripheral parts of the cell cytoplasm cells varying in size and sometimes adopting a rosette like formation. Giant cells may occasionally be noted. Fatty change and the alcoholic hyaline of Mallory are

not seen. There is considerable infiltration with predominantly lymphocytes but also plasma cells both in the portal zones and also in clusters among the liver cells. If autopsy material alone is studied the acute picture may have strikingly decreased and the picture may then be of a non specific post necrotic cirrhosis. In most instances however search will reveal the areas of focal necrosis and cell infiltration characteristic of this form of active chronic hepatitis.

Associated diseases. About half the patients seem to suffer in addition from conditions apparently unrelated to the liver. Arthralgia affecting the hands and larger joints appears in the particularly active case usually with fever. Skin rashes of various types may be seen apart from acne. These include erythema, purpura and face lesions indistinguishable from those of systemic lupus erythematosus.

The relation to ulcerative colitis is

term active juvenile cirrhosis of Reid and coworkers is therefore a misnomer and the title "Cirrhosis of young people" of Willock and Isselbacher is not always appropriate.

In the present series 70% were female (fig 2) but the occurrence in males must be emphasised and makes the title "Cirrhosis of young women" used by Beern and coworkers not always true. Nevertheless, the greater incidence in females contrasts strongly with the alcoholic group. In the cryptogenic group too, males are somewhat in excess (fig 2).

The onset is usually insidious, the patient feeling generally off colour and is then noted to be jaundiced. In some the condition presents as a clinically quite typical attack of acute viral hepatitis and this aspect will be discussed later. Icterus is a persistent feature the depth is variable the serum bilirubin level usually lying between 2 and 10 mg/100 ml. This persistent jaundice of hepatocellular type is a point of distinction from most other varieties of cirrhosis. The alcoholic is jaundiced in episodes rather than persistently and the cryptogenic type of cirrhosis is not usually associated with continued jaundice. As Wildenstrom emphasised amenorrhoea and spider naevi are usual and rene is prominent in adolescence. Splenomegaly is common, the liver is of variable size. In spite of the jaundice the biochemical disturbance and hepatic histology, the patient looks remarkably well and is strongly built. Hepatic precoma, ascites and portal hypertension are late features becoming

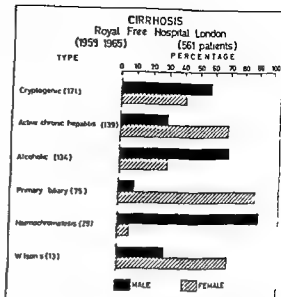


Fig 2 The sex distribution of cirrhosis in the Royal Free Hospital. Note that 70 of the 139 patients with active chronic hepatitis were female.

overt only after some two to four years of illness.

Biochemical The picture is of very active disease. Apart from the hyperbilirubinaemia of both conjugated and unconjugated type the gamma globulin levels are very high. Serum albumin is maintained until the later stages of clinical liver failure. Serum transaminases are very high. Towards the end transaminases and gamma globulin levels fall.

Hepatic histology (figs 3, 4) The findings on needle biopsy of the liver are usually characteristic. It is however not infrequent to encounter a persistent and profound increase of the prothrombin time unresponsive to intramuscular vitamin K therapy. This precludes liver biopsy.

The picture is of aggressive fibrosis, isolation and death of small groups

Treatment

Corticosteroid drugs inhibit immune and inflammatory mechanisms and may even inhibit virus replication. They might therefore be expected to benefit these patients. Administration does indeed give relief from fatigue, decreases jaundice and reduces the serum transaminase levels. The effect on serum gamma globulin values is less dramatic. The effect on the underlying histological changes in the liver is difficult to determine for there is such a great difference in the appearances in different parts of the same liver. Serial liver biopsies before and after corticosteroid therapy are difficult to evaluate but for what they are worth have shown that in terms of hepatocellular damage and cellular infiltration about an equal number have improved, worsened or remained the same. Once a true nodular cirrhosis has developed restoration of the liver to normal will be impossible whatever the treatment. The evolution of portal hypertension and of liver failure are inevitable although in the individual case these may come sooner or later. The life expectancy is probably little changed by corticosteroid therapy. Twenty six patients with this disease died. The mean duration from onset of symptoms in 12 who had received corticosteroid therapy was 3.1 years and in the 14 who had not was 1.5 years (Reid et al 1963). Infections are more frequent in the corticosteroid treated group and the facial disfigurement and bone thinning are among the other disadvantages. It seemed just

ifiable therefore to conduct a controlled clinical trial of the value of corticosteroid therapy. This is now being done at the Royal Free Hospital (Cook and Sherlock 1966). It commenced in 1963 and so far 30 patients who had never received previous corticosteroid therapy have been included in random fashion. Sixteen in the control and 14 in the treated group. One or two years later three of the controls are dead and three of the treated group all from liver failure or portal hypertension. With regard to symptomatic benefit the cortisone treated patients seemed to be in better general condition and more able to lead a normal life. This trial is clearly not complete and detailed analysis of the biochemical and histological differences between the two groups has not yet been made. Further patients are being included and more time must elapse before the place of prednisone therapy can be assessed. At present prednisolone should be given to those who are obviously ill, deeply jaundiced and unable to lead a normal life. The maintenance dose varies between 10 and 20 mg daily. Every six months attempts should be made to withdraw the drug but this usually proves impossible.

Other forms of immunosuppressant therapy must be considered. 6-mercaptopurine (6-MP) inhibits antibody production in experimental animals and has been used to treat patients with this type of active chronic hepatitis (Paine et al 1964). Four patients were treated in a dose of 1.5 mg per kg body weight and thus reduced jaun-

interesting, the bowel condition appearing before or after the active chronic hepatitis is diagnosed (Holdsworth et al 1956)

The kidneys are involved in a surprising proportion of cases even though routine assessment of kidney function by albuminuria, urinary deposit and creatinine may be normal. This may be shown only by renal biopsy. Twelve patients had needle biopsies of the kidney, two were normal, seven showed a glomerulitis of the type seen in systemic erythematosis, one had pyelitis, one nephrosclerosis and one hypokalemic nephropathy (Silva et al 1965)

Pulmonary infiltrates and areas of local collapse may be seen (Crispin and Lessof, 1965). Primary pulmonary hypertension has also been described (Colien and Mendelow, 1965)

Other associations include Hashimoto's thyroiditis, diabetes mellitus, rheumatic heart disease and thrombocytopenic purpura (Reid et al 1963)

Aetiological factors In about a quarter of the patients the illness seemed to begin with a typical attack of viral hepatitis. In some there was even a history of preceeding contact with the disease or of occurrence in an epidemic. This makes the association more possible. In others however the diagnosis of viral hepatitis remains presumptive. A similar histological picture in the liver can be seen among patients in a definite large epidemic of hepatitis. The likelihood is that acute virus hepatitis is one initiating factor in this active chronic hepatitis of Woldenstrom

The relation to disturbed immunity has aroused increasing interest starting with the observation of Joske and King (1955) of a positive L.C. cell phenomenon and continuing with the observations of Mackay and co-workers who used the term "lupoid hepatitis". The L.C. cell was in fact seen in only about 15% of patients usually in those in a particularly active stage with arthralgias and fever (Reid et al 1963). Apart from the activity there is no essential difference in those with a positive L.C. cell and those without. Bouchier and co-workers (1964) found other serological tests to be positive, of the antinuclear factor in 42% the latex fixation in 21% and the sheep cell agglutination in 53%.

Mackay (1961) suggested that liver cell components might become antigenic after viral or nutritional injury and so provoke an autoimmune reaction that is responsible for perpetuation of inflammation and progression to postnecrotic cirrhosis. The dominant lymphocytic and plasma cell infiltrate in the liver might support this theory. These cells might represent "forbidden clones" which are able to damage neighbouring tissue because of their antigenicity. Unfortunately destruction of liver cells is a response of this autoimmune process has never been confirmed and this hypothesis remains unproven. However the widespread involvement of various organs and the striking immunological disturbances leave no doubt that this is a systemic disease and not one of the liver alone.

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dice and transaminase levels. These events occurred long before any decrease in the serum gamma globulin values. This suggested that the immunological reactions seen in these patients are not the cause of the liver disease but rather secondary to destruction of liver tissue by an unknown aetiological agent or a reaction to the aetiological agent itself. One patient, after a year's treatment became resistant suggesting that the drug was proving unsuitable for long term use. However, Page and co-workers remarked that this therapy does not have the drawbacks of corticosteroids. The disadvantages are of leukopenia and marrow aplasia and of infections. Further evaluation of this treatment is necessary.

Prognosis This is extremely variable and at the outset unpredictable. Death is most frequent during the first four years after the recognition. Longer survivals, even up to 16 years may be seen. It may be speculated that many of the cases of cryptogenic cirrhosis may start as active chronic hepatitis. This surmise will be difficult to confirm for at autopsy, even in patients where the original hepatic biopsy showed active chronic hepatitis, the appearances are of a non-specific postnecrotic (macronodular) cirrhosis.

Summary

One hundred and thirty nine patients with the Active Chronic Hepatitis of Waldenström were seen among 561 patients with cirrhosis of the liver seen at the Royal Free Hospital between

1959 and 1965. 48 % were aged between 10 and 29 years and 70 % were female. Clinically, although persistently jaundiced to a varying degree, the patients remained relatively well until the terminal stages. Anaemorrhoea was usual in the younger women. Biochemical changes included high gamma globulin and transaminase levels. Hepatic histology showed a very active picture with plasma and lymphocytic infiltration, aggressive fibrosis with isolation of liver cells into small groups and a postnecrotic cirrhotic

Associated diseases were frequent and included ulcerative colitis, skin rashes, glomerulitis, pulmonary infiltrates, diabetes and Hashimoto's disease. Only some 15 % showed L.F. cells in the blood.

Aetiology is mentioned and the place of corticosteroids to relieve symptoms and jaundice is discussed.

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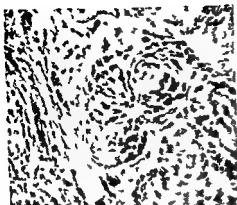


Fig 1 Liver biopsy performed in October 1969. Prussian blue stain for iron magnification $\times 60$. Iron is present both in macrophages in the bands of fibrous tissue and in parenchymal cells in regenerative nodules. With repeated phlebotomy over a three year period there was a complete disappearance of stainable iron in the liver.

nocturia, weight loss, palpitations or dyspnea on exertion. At the time he had glomerulonephritis in 1960 his liver was palpable 2 cm below the costal margin but tests of liver function were normal. There had been no increase in skin pigmentation. There was no family history of liver disease or anemia. The patient was not anemic and had a normal reticulocyte count. Serum iron determinations were 18 and 120 $\mu\text{g}\%$ with 100% saturation. Iron binding protein. Liver function was normal except for BSP retention of 17.5% (Table 1) and an electrocardiogram was normal. Although fasting blood sugar levels were normal a oral glucose tolerance curve suggested latent diabetes.

Since November 1969 the patient has been treated by repeated phlebotomy. With removal of 40 liters of blood the liver deposits of iron have been completely mobilized, serum iron concentration has fallen and the patient has become slightly anemic. In October 1969 liver function tests were within normal limits (Table 1), the electrocardiogram remained normal and a glucose tolerance test was normal. The urinary excretion of ketosteroids

Table 1 Laboratory findings

	1963		1961		1960	
	Oct-Dec	January-December	January-December	January-October	January-October	January-October
Normal eryth						
Serum iron	98	40	34	40	34	33
Iron binding capacity	185	10	19	10	19	10
Iron in blood	185	10	21	10	21	10
BSP retention (15 min)	0.306	0.206	0.206	0.10	0.10	0.02
BSP retention (15 min)	17.5	27	27	41	41	10
Alkaline phosphatase	40	37	37	44	44	41
Cholesterol (mg/dl)	30	27	27	34	34	32
Alkaline phosphatase (BUN)	33	26	26	34	34	17
SCOT		28	28			

Articular Chondrocalcinosis in a case of Hemochromatosis

By GÖRAN C. H. BAUER and GRAHAM H. JEFFRIES

I Introduction

Articular chondrocalcinosis is a rare condition characterized by generalized calcification of cartilage. This condition may involve articular or intra-articular cartilage, including menisci, intervertebral discs and the pubic symphysis. Articular chondrocalcinosis has been described both as an isolated entity (13), and in association with several metabolic diseases, by hyperparathyroidism (3), Wilson's disease (1), ochronosis (8) or hemochromatosis. Cases of hemochromatosis with chondrocalcinosis were first described by de Seze et al. in 1964 (11) and four additional cases have subsequently been published by other French authors (4, 6).

The case described here had arthralgia and radiographic evidence of chondrocalcinosis at least two years before hemochromatosis was diagnosed.

II Observations

A 59 year old plasterer was referred to the Orthopaedic Out Patient Clinic at The Hospi-

tal for Special Surgery because of pain in the right knee. The patient had been treated for hemochromatosis by repeated phlebotomy since 1962. X-ray revealed generalized articular chondrocalcinosis. Arthroscopy with removal of the medial meniscus was performed.

A Medical History Prior to Diagnosis of Hemochromatosis

The patient had been well until 1960 when he developed acute glomerulonephritis diagnosed on the basis of fever, hypertension, edema, hematuria, albuminuria and renal biopsy. He recovered completely from this illness without residual hypertension or evidence of chronic glomerulonephritis.

B Diagnosis and Treatment of Hemochromatosis

In 1962 the patient was admitted to The New York Hospital because of a tender enlargement of the right breast. Microscopic examination of excised breast tissue confirmed a diagnosis of gynecomastia. An elective cholecystectomy was performed in October 1962 for asymptomatic radio-opaque gall bladder calculi. At the time of operation the liver was found to be enlarged and cirrhotic and a biopsy revealed typical hemochromatosis (Fig. 1). In the subsequent assessment of this problem it was found that the patient had been impotent for the previous 8 months but had suffered from no polyuria, polydipsia



Fig 4 X-ray of medial semilunar cartilage removed from right knee

siderosis. The meniscus showed degeneration of fibrocartilage and many deposits of birefringent calcific material in its substance (Fig 5). Similar deposits were present in the articular cartilage of the femoral condyle.

4. X-ray diffraction (E. D. Lanes Ph.D.) of a fragment of the bony mass removed from the antero-medial compartment revealed the presence of randomly oriented microcrystals of a mineral apatite and failed to show evidence of any distinct crystalline pyrophosphate.

5. Blood chemistry. Latex fixation tests in 1960, 1961 and 1963 were negative. Serum calcium and phosphorus and alkaline phosphatase analyses in 1960, 1963 and 1963 were normal. Serum uric acid analyses performed in 1960 were reported as 7.3, 4.8, 7.1, 4.8 and 5.0 mg per 100 cc and in 1963 4.4 mg per 100 cc. Only two values were thus higher than the normal of 6.0 mg per 100 cc.

III Discussion

It is well known that synovial deposition of hemosiderin occurs in hemochromatosis, but this manifestation of the disease has never been shown to produce clinical signs of arthropathy. However, recently de Sèze et al. (11) published 6 cases. Delbarre (4) 2 cases and Jaffres and Kerbrat (6) 2 cases of

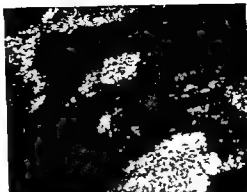


Fig 5 Section of semilunar cartilage showing birefringent deposits. Hematoxylin-eosin stain viewed in polarizing microscope magnification $\times 144$.

hemochromatosis with arthralgia and articular chondrocalcinosis diagnosed radiographically. The case published here is particularly interesting because chondrocalcinosis was shown to be present prior to the onset of symptoms attributable to hemochromatosis and because analyses of cartilage, bone and one meniscus showed the presence of calcium deposits without iron. However, the synovium contained iron whereas the liver no longer did. This was found also in the case reported by Kra et al. (7).

Although the association of articular chondrocalcinosis with hemochromatosis has only recently been recognized, this is probably not rare. By systematic radiographic examination of the joints in 6 cases of hemochromatosis de Sèze et al. (11) found 2 cases of chondrocalcinosis and Schumacher (9) found 5 cases of arthralgia in a review of 23 cases of hemochromatosis. Arthralgia in patients with hemochromatosis may be neglected be-



Fig 2 X ray of right hip in 1965 Calcification of articular cartilage produces a line concentric to the bone

roids was 7.0 mgm in 24 hours (normal for male 15 mgm per 24 hour urine)

C Arthralgia and Diagnosis of Chondrocalcinosis

The patient has suffered from intermittent pain in several joints including the right shoulder and both knees since 1958. In 1960 when he was admitted with glomerulonephritis he complained of pain in the knees and both joints were tender, warm and swollen.

In 1960 the patient was referred for orthopaedic care because of pain in the right knee of several months duration. There was no history of trauma. He walked with a slight limp and he could not perform a deep knee bend. The right knee had a loss of 10° full extension, slight effusion, a palpable mass in the medial compartment anteriorly, slight ten-



Fig 3 X ray of right knee in 1965 Both semilunar cartilages are visible because of calcification

derness on palpation of the medial joint line but was otherwise normal on physical examination as were all other joints.

1 X ray examination of the joints in 1965 revealed generalized cartilage radiodensity, especially apparent in shoulder, hip, knee joints and in the semilunar cartilages in the intervertebral discs of the lumbar spine, in the pubic symphysis and in the triangular cartilage of the radiocarpal joints but not present in the finger joints. Review of x rays of 1960 revealed that this condition was present even at that time, i.e. two years prior to the diagnosis of hemochromatosis (Figs 2 & 3).

2 Arthrotomy of the right knee joint with removal of the medial meniscus was performed because of the extension defect and tenderness at the medial joint line. The joint contained some 50 cc of viscous, dark yellow, clear fluid. The synovium was not thickened and had normal color. The cartilage of the femoral and tibial condyles was encrusted with a white material and in a few spots white hard material was deposited on the cartilage surface. Both menisci were likewise encrusted (Fig 4); the medial meniscus was frayed at its central border. A bony hard piece of tissue 1 x 1 cm large was attached to the anterior horn of the medial meniscus.

3 Histologic examination (Robert C. Melchers, M.D., Ph.D.) of the synovium showed deposits of hemosiderin in synovial cells and macrophages, diagnostic of synovial hemo-

other radiographic signs of chondrocalcinosis hemochromatosis should be considered as a possible diagnosis

Acknowledgement

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cause of the more severe manifestations of this disease. Advanced chondrocalcinosis, however, may cause no symptoms, our patient has no symptoms of hip disease in spite of his radiographic signs.

From the published case reports the menisci appear to be the most common site of chondrocalcinosis in hemochromatosis (4, 6, 11), as in idiopathic chondrocalcinosis (13). In one of Schumacher's (9) two cases and in a case published by Kraetzel (7) meniscal calcifications were noted even though chondrocalcinosis otherwise was not discussed in these two publications. Calcified menisci are rarely found in any of the common arthritides. Delbarré (4) and Hoskin and Glenister (5) quote three authors who found meniscal calcifications in about 0.3% of radiograms of the knee. Five of 8 cases reported by one author (12) had radiographic evidence of chondrocalcinosis of other joints. X-ray evidence of meniscal calcifications is thus highly suggestive of chondrocalcinosis and whenever this is seen the associated metabolic diseases should be considered in the differential diagnosis.

The etiology of chondrocalcinosis in hemochromatosis is not known. The absence of iron deposits in the cartilage in our patient and the presence of trace amounts only in a patient described by de Seze et al (11) suggest that the local accumulation of iron is not important. By way of speculation attention should perhaps be drawn to Selye's (10) experimental studies of calcification, he was able to precipitate

tissue calcium deposits by local injections of iron and other metal salts. However, this was possible only after administration of toxic doses of vitamin D.

Bundens et al (2) have recently stressed that in cases of idiopathic articular chondrocalcinosis with arthritis ("Pseudogout Syndrome") the joint fluid contains calcium pyrophosphate identifiable by x-ray diffraction. In the case described here the joint fluid did not contain any crystals, and x-ray diffraction of an intra-articular calcific deposit suggested the presence of calcium apatite rather than calcium pyrophosphate.

IV. Summary

A 59 year old man had arthropathy and radiographic evidence of generalized chondrocalcinosis two years before hemochromatosis was diagnosed. During a three year period repeated phlebotomy has completely removed iron from the liver and the patient has developed no further complications of hemochromatosis.

At this time knee arthrotomy with meniscectomy was performed. The articular cartilage and meniscus contained calcium but not iron. X-ray diffraction of calcific deposits suggested the presence of apatite rather than pyrophosphate structure.

Comparison with previously published cases of chondrocalcinosis associated with hemochromatosis indicates that this association may not be rare though described only recently. In patients with meniscal calcifications or

Table I Age and sex distribution of present SLE series and of two American SLE series

Series	Age at diagnosis years								Total	Sex	
	5-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79		Males	Female
Present series	0	1	10	16	13	5	7	2	54	6	48
%	0.0	1.8	18.5	29.6	24.1	9.3	13.0	3.7	100.0	11.1	88.9
S of Merrill and Shulman	0	10	20	29	27	7	6	0	99	13	86
%	0.0	10.1	20.2	29.3	27.3	7.1	6.0	0.0	100.0	13.1	86.9
S of Hellum and Haverick	3	47	81	70	55	22	18	8	299	42	257
%	1.0	15.7	27.1	23.4	18.4	7.4	6.0	2.7	100.0	14.0	86.0

Table II Data for calculation of survivorship present SLE series

Yr after diagnosis	Nr observed x or more Yr after diagnosis	Nr last observed and alive in interval between this x and next stated x Yr	Nr dying in interval between this x and next stated x Yr	Estimated proportion of persons surviving to this x who		Percentage of persons diagnosed who survive x Yr
				die before next stated x Yr	survive to next stated x Yr	
(x)	(O _x)	(l _{awx})	(n _{dx})	(a _{px})	(a _{qx})	(P _x)
(1)	(2)	(3)	(4)	(5)	(6)	(7)
0	54	0	10	0.185	0.815	100.0
1	44	0	2	0.045	0.955	81.5
2	42	0	3	0.071	0.929	77.8
3	39	1	0	0.000	1.000	79.3
4	38	5	1	0.026	0.974	79.3
5	32	8	2	0.063	0.937	70.3
6	28	4	1	0.036	0.964	65.7
7	22	4	1	0.045	0.955	63.2
8	18	4	1	0.063	0.937	60.2
9	13	4	1	0.091	0.909	56.4
10	8	3	1	0.154	0.846	51.3

tively) where is the present material was composed only of Scandinavians. All patients except three had been treated with corticosteroids (94.6 %) compared with 74.8 % of the patients of Merrill and Shulman and 90.0 % of the patients of Hellum and Haverick.

The data for calculation of survival are given in Table II. Column 5 represents the estimated mortality for each year after diagnosis and column 7 the cumulative survival percentages. It is seen that 70.3 % of the patients were expected to survive for five years and

Long-Term Prognosis of Systemic Lupus Erythematosus

By TORE LEONHARDT

Statistical evaluations of the long term prognosis of systemic lupus erythematosus (SLE) have been published by Merrell and Shulman (1955) and by Kellum and Haserick (1964). Since no such study seems to have been made of European materials of SLE, it was considered worth while to present the results of a follow up of 54 Swedish patients with SLE.

Material and methods

The material consists of a series of patients with SLE seen at hospitals in Scania 1955–1961. It was originally collected to study genetic factors in the etiology of SLE (Leonhardt 1964). Perusal of registers of hospital inpatients, biopsy and necropsy files of departments of pathology and LE cell registers of hospital laboratories revealed 113 cases with a clinical picture consistent with SLE. Of these 59 were considered to have "definite" SLE having at least one diagnostic criterion in the form of "pathognomonic" necropsy findings characteristic skin changes or a positive LE cell phenomenon and a medical history judged as typical of SLE. The remaining 54 cases had a less firm diagnosis and were not included in the present study since they were not thought to be comparable with the SLE patients selected by the above mentioned authors.

In five of the 59 cases with definite SLE the diagnosis was not established until post

mortem. These cases were not relevant to a study of long term prognosis and were therefore excluded. In the remaining 54 cases the duration of the survival was calculated from the date of diagnosis to death or date of last observation of those still living. The diagnosis was taken as the starting point because it is difficult to date the onset of SLE in a given case (Merrell and Shulman 1955, Leonhardt 1964). The follow up was discontinued in November 1965.

The statistical method described in detail by Merrell and Shulman (1955) and also used by Kellum and Haserick was chosen for the present study. The probability that a person alive in a stated year after diagnosis will die before the next year was estimated by application of the following formula to the data (symbols see Table II).

$$nq_x = \frac{nd_x}{O_x - 1/nw_x}$$

Results

Of the 54 SLE patients six (11.1 %) were males, the material thus showing the usual preponderance of SLE in females (Table I). The age distribution is also shown in Table I. In the series of Merrell and Shulman as well as in the series of Kellum and Haserick the patients were on the whole younger. Both American series included Negroes (26.3 % and 7.7 % of total respect

Table 1 Age and sex distribution of present SLE series and of two American SLE series

Series	Age at diagnosis years								Total	Sex	
	5-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79		Males	Females
Present series	0	1	10	16	13	5	7	11	54	6	48
%	0.0	1.8	18.5	29.6	24.1	9.3	13.0	3.7	100.0	11.1	88.9
S of Merrell and Shulman	11	10	20	29	27	7	6	0	93	13	86
%	0.0	10.1	20.2	29.3	27.3	7.1	6.0	0.0	100.0	13.1	86.9
S of Kellum and Haverick	3	47	81	11	22	18	3	299	42	257	
%	1.0	10.7	27.1	2.34	18.4	7.4	6.0	1.0	100.0	14.0	86.0

Table II Data for calculation of survivorship present SLE series

Yr after diagnosis	Nr observed x or more Yr after diagnosis	Nr last observed and alive in interval between this x and next stated x Yr	Nr dying in interval between this x and next stated x Yr	Estimated proportion of persons surviving to this x who		Percentage of persons diagnosed who survive x Yr
				die before next stated x Yr	survive to next stated x Yr	
(1)	(O ₁)	(a _w ₁)	(a _d ₁)	(a _p ₁)	(a _q ₁)	(P ₁)
(1)	(2)	(3)	(4)	(5)	(6)	(7)
0	54	0	10	0.185	0.815	100.0
1	41	0	2	0.049	0.951	81.5
2	47	0	3	0.071	0.929	77.8
3	39	1	0	0.000	1.000	72.3
4	33	5	1	0.028	0.972	72.3
5	32	2	2	0.063	0.937	70.3
6	29	4	1	0.033	0.967	63.2
7	23	4	1	0.018	0.982	63.2
8	18	4	1	0.063	0.937	60.2
9	13	4	1	0.091	0.909	56.4
10	8	11	1	0.154	0.846	51.3

tively) whereas the present material was composed only of Scandinavians. All patients except three had been treated with corticosteroids (94.6 %) compared with 75.8 % of the patients of Merrell and Shulman and 90.0 % of the patients of Kellum and Haverick.

The data for calculation of survival are given in Table II. Column 5 represents the estimated mortality for each year after diagnosis and column 7 the cumulative survival percentages. It is seen that 70.3 % of the patients were expected to survive for five years and

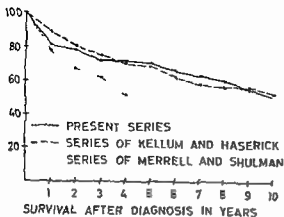
SURVIVAL
PER CENT

Figure 1 Survival of patients with systemic lupus erythematosus

51.3 % for ten years after diagnosis. Similar figures were found by Kellum and Haserick (69.4 % and 53.7 % respectively), while Merrell and Shulman observed a higher mortality only 51.5 % of their patients were estimated to survive for four years after diagnosis (the patients not being followed long enough to calculate the five or the ten year survival rate). The estimated survival rates calculated for the three materials in comparison are given in a graphic form in Figure 1.

The high mortality during the first year after diagnosis in all three materials is due to the fact that many patients were not hospitalised and diagnosed until the acute onset of a severe SLE crisis. In the subsequent years the annual mortality was significantly lower and relatively constant.

In most diseases, survival is influenced by the composition of the material as to sex, age and other variables. According to Kellum and Haserick the prognosis of SLE is poorer among

males than among females. The course of SLE is held to be particularly unfavourable in children (Cook et al 1960). On the other hand, aged patients run an increased risk of being affected by other diseases which lower vitality. Racial differences in course and outcome of SLE may exist, but hitherto published data (Kellum and Haserick 1964) are inconclusive. Differences with regard to such variables make strict comparisons between the three mentioned SLE series impossible. It is however interesting to note the close survival curves and the almost identical figures for five and ten years survivorship in the present series and in the series of Kellum and Haserick.

The data of Kellum and Haserick indicated that the prognosis of SLE may be improved by corticosteroid treatment, although the small number of untreated patients did only permit any valid conclusions for the first two years after diagnosis. Thus the higher mortality found in the series of Merrell and Shulman than in the present series and in that of Kellum and Haserick might, at least partly, be explained by the fact that one fourth of their patients did not receive hormone treatment against only five and ten percent respectively, of the patients in the other two series.

The survivorship figures must be interpreted in the light of the general death rate in the population from which the patients derived. According to the 1958 life tables in Sweden (Statistisk Årsbok för Sverige 1964), the survival rate of a group of the Swedish population matched to the present

SLI series as to sex and age would be 97.3 % after five and 93.6 % after ten years.

Although the introduction of corticosteroid treatment has made it possible to influence the course of SLI favourably SLI must still be regarded as a serious disease with a dubious long term prognosis. Only about half of the patients in a series of definite SLI cases can be expected to survive for ten years after diagnosis.

Summary

The long term prognosis of systemic lupus erythematosus (SLE) was studied in 64 Swedish patients. The estimated survival after diagnosis was 70.3 % after five years and 61.3 % after ten years. The life expectancy of a sex

and age matched Swedish population was 97.3 % and 93.6 % respectively. The results were compared with the survival rates in two American SLE series.

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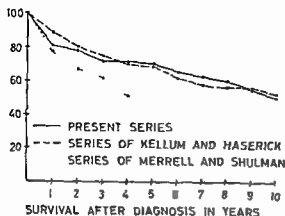
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Fig 1 Peribronchovascular bundles packed with eosinophil cells



Fig 3 Eosinophilic granuloma (arrow) in the peribronchovascular tissue



Fig 2 Transgression of the vessel wall by eosinophils which are scattered in the mucosa



Fig 4 Marginal emphysema and eosinophilic infiltration of the lung

Polyangitis in Allergic Conditions

By P. KALLOS and L. KALLOS-DEFFNER

In a stimulating paper, presented at the Second Symposium of the Collegium Internationale Allergologicum in Basle in 1956, J. Waldenström stated that more or less widespread periarthritis nodosa ("polyangitis") has, at least in a certain number of cases, an allergic etiology. Furthermore, he described enlightening case histories, showing that spontaneous remissions of polyangitis are "by no means rare." Intensive treatment with corticosteroids can have either a favourable temporary effect, or cure even long lasting and severe cases of this condition.

At autopsy of asthmatic patients who died in status asthmaticus, the occurrence of periarthritis nodosa was first noted by E. Böhrmann in 1935. Later many such cases were described and we refer to the review by E. Churg and L. Strauss. Recently K. F. W. Hinson claimed that arteritis (excluding areas of infection and infarction), i.e. necrosis of the vascular wall and a local inflammatory response with intense eosinophilia can be found at autopsy in asthmatics. He

is inclined to suppose that Löfller's eosinophilic infiltration (cf. 10, 11) allergic granulomatosis, Wegener's syndrome and polyarthritis nodosa are 'imperceptibly merging conditions of increasing severity'.

The examples given in Waldenström's paper allow the assumption that febrile episodes with blood eosinophilia in the course of asthma, drug allergy and other allergic conditions are the first symptoms of polyangitis. These can have a grave prognostic significance (15, 3).

In experimental allergic asthma in guinea pigs induced by inhalation of the aerosolized specific antigen we could show (5, 6, 7, 8) that already after one severe asthmatic attack of short duration (3—10 minutes) the bronchial vessels are packed with eosinophil cells (fig. 1). After some consecutive attacks (3—5 inhalations) the eosinophils infiltrate and transgress the vascular wall and invade the surrounding tissue (fig. 2). The eosinophils form granulomas (fig. 3) or after several asthmatic attacks (up to ten) marginal infiltrations in the lungs (fig. 4).

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The lungs show, at the same time, emphysematous and teleclastic changes, resembling those recently described by J G Leopold in humans. These are certainly allergic changes. Already in 1937 we could show (8) that the inhalation of histamine- or acetylcholine aerosol in guinea pigs induces the clinical picture of asthma but never the above described histological changes (cf also 12). As we (5, 6, 7, 8) and W Pagel (13) pointed out, in these asthmatic guinea pigs not only the lungs but other organs such as the heart and spleen show similar changes which must be considered as the first stages of allergic polyangitis. In spite of their severity and extent, these experimentally induced changes in asthmatic guinea pigs are completely reversible. Spontaneous recovery occurs in about 30—50 p C of guinea pigs exposed at least ten times "Tuberculoid" granulomas, infection and scars occur then in the affected tissues.

Stimulated by the clinical observations of J Waldenström we could show (4 and unpublished observations) that by carefully planned treatment with corticosteroids the rate and speed of recovery of asthmatic guinea pigs can be enhanced considerably. We also pointed out as J Waldenström did the close connection of the described changes with alterations of the connective tissue. This constitutes a link between this group of allergic conditions and the so called connective tissue- or collagen diseases. In the latter polyangitis is a common feature (14). In circumscribed and extremely in-

tense biphasic allergic lesions ("anaphylactic myositis") and as a sequel of challenging allergic animals simultaneously with the specific antigen and vasoactive drugs (such as caffeine) all features and stages of allergic polyangitis could be induced by us (7, 13, 14).

These cursory remarks are intended to show that the careful observations of J Waldenström provided a clinical counterpart to experimental findings and opened new avenues for clinical therapy on one hand and experimental work on the other. We consider this typical of his work which enriched so many different fields of medicine.

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Waldenstrom's Uveoparotitis

By D. GERAINT JAMES

M.A. M.D. (Cantab) F.R.C.P. (London)

Thirty years ago Jan Waldenstrom (1) submitted for publication in *Acta Medica Scandinavica* an account of his observations of patients with uveoparotitis attending the medical surgical and ophthalmological clinics in Uppsala. He described five patients with bilateral parotid gland enlargement and bilateral uveitis. Four of them were women and they were all over 30 years of age. The disease process was clearly multisystem for one or other of these patients had evidence of involvement of the neurological system, lungs, lymph nodes or skin as well as fever or hyperglobulinemia. Sarcoid tissue was clearly demonstrable in the parotid gland tissue of one patient. Waldenstrom drew particular attention to bizarre neurological manifestations of sarcoidosis which included right sided facial palsy in two patients and a right hypoglossal nerve palsy or bilateral optic neuritis once apiece. One patient "was regarded as a case of encephalitis lethargica with signs of involvement of the brain stem (lethargy), the cerebral cortex (hallucina-

tions) the pyramidal tract (positive Babinski reflexes), the sensory system (impaired deep sensibility in the toes and other disturbance of sensibility) the cerebellum (positive sign of Romberg, intention tremor, signe de fren) and of the peripheral neurone (abolished patellar and achilles reflexes, peripheral facial palsy). At the same time there was conclusive evidence of meningeal involvement (pleocytosis, increase in albumen and pathological mastix curve).

The significance and importance of this publication can only be evaluated in the light of current thinking at that time. Heerfordt (1909) (2) had already drawn attention to the syndrome of "Febris uveoparotidea subchronica" characterised by uveitis and enlargement of the parotid glands running a chronic and usually febrile course and frequently complicated by cranial nerve palsies (especially of the seventh cranial nerve with pleocytosis of the cerebrospinal fluid). Heerfordt described three cases and referred to other examples he had found in the literature. Since some of these latter

veral causes of enlargement of the parotid gland is by seeking corollary evidence in other tissue systems and by obtaining histological proof of sarcoid tissue. In a recent series (3) enlargement of the parotid gland was observed in 23 of 388 (6%) patients with histologically-confirmed sarcoidosis. The most frequent clinical accompaniments are enlargement of spleen and lymph nodes, uveitis and abnormal chest radiographs, whilst histological confirmation is most conveniently obtained by biopsy of lymph node, skin, liver, parotid or by means of the Kveim test. Sarcoid parotitis may be acute, transient and self-limiting, or it may be chronic and persistent. Corticosteroids seem unnecessary in the transient group and provide no clearcut benefit in chronic persistent enlargement of the parotid

gland. However, steroids have influenced the natural history of uveitis and are always indicated, either topically or parenterally, to control this otherwise distressing and crippling manifestation of uveoparotitis (4).

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Radiovitamin B₁₂ as a biological reference substance III Glomerular filtration rate

B. P. O. GRANBERG and PETER REIZENSTEIN

Vitamin B₁₂ is one of the few physiologically occurring organic molecules which can be labeled with a gamma emitting radio isotope without changing the molecule itself. The localization of the cobalt atom centrally in the cobalamin molecule provides for an extraordinary stability of the label. No liberation *in vivo* of the radio cobalt from the cobalamin has been demonstrated (1, 2). In addition vitamin B₁₂ can be labeled with four different radioactive cobalt isotopes and the substance is quite non toxic. No instances of hypersensitivity to vitamin B₁₂ have been reported. These facts render radiovitamin B₁₂ unusually suitable for the use as a biological reference substance in the physiological and chemical studies of different organs. The two first parts of this project involved the use of radiovitamin B₁₂ quite successfully in the study of gastric function (3) and with rather less success in the study of hepatic function (4).

The purpose of the present work is to examine the possibility to use radio vitamin B₁₂ in the study of renal function i.e. to compare glomerular filtration rates obtained with radio B₁₂ to those obtained with inulin. The basis for this idea was the observation that renal disease affects the result of the urinary vitamin B₁₂ excretion test in diagnostic studies of hematologic conditions (5).

During the progress of this work two articles have appeared (6, 7) showing good agreement between the glomerular filtration rate measured with inulin and that measured with vitamin B₁₂. These results are confirmed and extended in the present paper.

Material and methods

Six patients were investigated and all together 34 clearance periods were studied with each method. The patients are described in table 1. The investigations were performed in the morning with patients in the fasting

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Table I *Description of patients*

Symbol	Age/sex	Diagnosis	No of clearance periods
A C	57/f	Stenosis of renal artery with normal kidney function	6
KAB	20/m	Volunteer control with normal kidney function ¹	11
KG	40/m	Volunteer control Normal kidney function ¹	11
SEN	33/m	Volunteer control Normal kidney function ¹	11
OW	72/m	Renal carcinoma on right side	6
GGT	38/m	Stenosis of renal artery with hypertension	4

¹ Patient treated with Octapressin® (phenyl alanine lysine vasopressine) after 2 control periods in the clearance study

state. The patients were hydrated before and during the investigation. The intravenous infusions of the clearance substances were made through a percutaneously introduced polyethylene catheter in a brachial vein. In order to saturate the B₁₂ binding capacity of the serum, a loading dose of 2 000 µg cyanocobalamin was given. The specific activity was 0.4 nc/µg and the ⁵⁷Co label was used. Simultaneously, 0.5 ml of a 10 per cent inulin solution per kg body weight was given. This prime dose was followed by an intravenous infusion with a constant speed syringe pump at a rate of 0.5 ml or 15 µg cyanocobalamin and 37.5 mg inulin per minute. This infusion was given during a 30 minute equilibration period prior to the first clearance period and continued during the entire test

Plasma samples for determination of inulin and B₁₂ were collected in the middle of each clearance period through a polyethylene catheter introduced in the brachial artery. These periods lasted from 15—20 minutes.

The bladder was evacuated with a Foley catheter *a demeure*. To minimize the dead space factor, the emptying procedure at the end of the periods was assisted by flushing with water and air as described earlier (8).

Inulin determinations in plasma and urine were performed colorimetrically at 530 mµ as described (9).

Radioactivity determinations were performed on 3 ml plasma or urine samples in a 2 1/2" × 3" well type NaI (Th) crystal with the help of a Gammatrix sample changer. Pulses amplified 250 times were recorded on an Ekco N 650 scaler pulse height analyzer and measurement times so adjusted, that the average and maximum measurement errors secondary to the random nature of the disintegrations were respectively 0.13 per cent and 0.71 per cent for urine and 2.7 per cent and 3.3 per cent for plasma.

Results

The deviations from the ideal constant concentration in the serum of radio B₁₂ and inulin respectively during the experimental period were studied and expressed as the mean of the standard deviation in per cent of the mean concentration for each individual patient. These percentages were 4.99 for inulin and 4.06 for B₁₂.

A statistically significant correlation

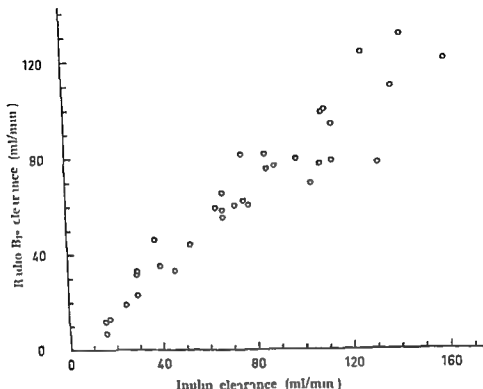


Fig 1 Correlation between glomerular filtration rate as measured with radio B_1 and with inulin

between the glomerular filtration rates determined with radio B_1 and with inulin was found

The correlation coefficient was 0.90 and the regression line for B_1 upon inulin was

$$B_1 = 5.155 + 0.764 (\text{inul})$$

The S.D. of the regression coefficient was 0.0442 and the regression coefficient thus differs from zero in a statistically significant manner ($p < 0.01$)

Discussion

Glomerular filtration rates are usually measured with the inulin clearance

method. Inulin is filtered in the glomeruli and neither secreted nor reabsorbed in the tubules. However, the customary colorimetric inulin determinations are rather laborious. Another disadvantage is that both glucose, fructose and certain drugs such as dextrane interfere with the inulin assay.

All these circumstances have made inulin clearance determinations laborious, difficult and rare. In clinical routine the endogenous creatinine clearance is therefore used to measure the glomerular filtration rate. However, the values thus obtained are ap-

proximate since there is both secretion and reabsorption of creatinine in the tubules. This renders the clearance values obtained with creatinine falsely too high in, e.g., uremic conditions.

It is our impression that radiovitamin B₁₂ has none of the disadvantages of inulin, and might provide a method of choice for determining the glomerular filtration rate.

Summary

Vitamin B₁₂ being a physiologically occurring organic compound with a gamma emitting isotope as a stable, natural part of the molecule, is suitable as a biological reference substance. In parallel studies of the glomerular filtration rate with inulin and radio B₁₂, a statistically highly significant correlation with a coefficient of 0.95 was found. Radio B₁₂ determinations had smaller errors than those of inulin and were much less laborious. Substances interfering with inulin assays do not interfere with the assays of radio B₁₂. The serum concentrations of inulin were less constant than those of radio B₁₂ which might pro-

vide a method of choice to measure the glomerular filtration rate.

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Pressure variations in the rectum and ileum during experimentally induced urgency of defecation

By FRANZ BARANY

One occasionally encounters diarrhea of obscure causation. This condition is usually classified as "functional diarrhea" and the course of the disease seems often to be influenced by psychological factors. The present study was undertaken to examine whether in such cases the mechanism of defecation presents any abnormality and if so to analyse it.

The series

The following groups of patients were chosen for this investigation:

14 control subjects evacuating once daily without discomfort, the stools being normal in shape and consistency.

4 patients with constipation who had not more than 2 spontaneous defecations a week. They had been instructed not to use laxatives for at least 3 days before the experiment.

11 patients with functional diarrhea diagnosed at this department all having at least 4 loose stools a day, one or more with imperative urgency. No

antidiarrheal drugs had been given for at least 2 days before the experiment. Achlorhydria, steatorrhea, pancreatic insufficiency, enteritis, colitis and intestinal stenosis and tumour had been excluded by thorough clinical and radiological examinations.

11 patients who at least one year before the experiment had undergone partial gastrectomy and Polya gastroenterostomy. 7 of them also bilateral truncal vagotomy. Two of the vagotomy patients had occasional diarrhea at irregular intervals and seldom lasting more than a day. Otherwise the patients in this group had 1—3 evacuations a day with stools of normal or almost normal consistency. None had diarrhea during the period of the experiment.

6 patients who at least one year before the experiment had had a partial resection of the rectum and/or colon with an end to end anastomosis. All had suffered from carcinoma of the bowel but at the time of the experiment were feeling well and there were no signs of metastases.

proximate since there is both secretion and reabsorption of creatinine in the tubules. This renders the clearance values obtained with creatinine falsely too high in, e.g., uremic conditions.

It is our impression that radiovitamin B₁₂ has none of the disadvantages of inulin, and might provide a method of choice for determining the glomerular filtration rate.

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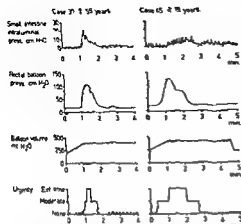


Fig 1 Controls. Urgency and contraction of rectum provoked by filling the rectal balloon then disappeared spontaneously although the balloon volume was kept constant. Pressure activity of small intestine synchronous with rectal contraction.

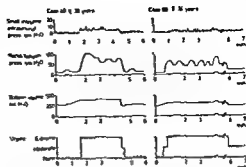


Fig 2 Functional diarrhea. No spontaneous disappearance of urgency or rectal contraction unless the balloon volume was reduced. Pressure activity of small intestine synchronous with rectal contraction.

1a—2a ml to the balloon or to decrease the volume by 2a—50 ml and then refill by the same amount.

The attacks of urgency had no regular effect on the propulsion of the endoradiosonde. In some instances its position was unchanged throughout the procedure while in others the sonde moved considerable distances before during and after an attack.

The 4 constipated patients displayed no deviation from the normal course.

In all the cases of functional diarrhea the rectum showed an inability to adapt itself to a filling that had provoked contraction. There was either an apparently tonic contraction of the rectum or a succession of rhythmic contractions and abortive relaxations. In neither case did the feeling of urgency or the provoked activity of the small intestine disappear unless

the volume of the rectal balloon was reduced. The reactions of 2 cases of functional diarrhea are illustrated in figure 2.

In the 7 patients that had had a partial gastrectomy, Polya gastroenterostomy and truncal vagotomy filling elicited a normal contraction of the rectum and feeling of urgency. One of them exhibited normal adaptation at most complete relaxation being recorded within 2 minutes. In the others relaxation was slower, taking more than 2 minutes during which several pressure waves of decreasing height were recorded (Figure 3). In 3 patients no pressure activity of the ileum was provoked during the attack of urgency. In 2 there was hardly any evidence of such activity. Only in the remaining 2 patients was a normal and distinct response of the small intestine obtained.

A completely normal response was found in 3 of the 4 subjects that had had a Polya operation without truncal

Methods

The intestinal motility was recorded by the endoradiosonde technique. The endoradiosonde Type P 2 (5) is sensitive to intraluminal pressure and transmits information to a receiving system outside the patient. The sonde is 9 mm in diameter, 16 mm long and weighs 2.3 g. The intraluminal pressure waves are plotted by an ink recorder. The movements of the endoradiosonde through the small intestine are charted by a tracking antenna system (4). The patient lies on a couch and a sheet of methacrylate 60×64 cm slightly curved and covered with a sheet of paper is placed a few centimeters above the abdomen. The movements of the antenna which follows the sonde are recorded by an ink pen on the paper.

The sonde was swallowed by the gastroenterostomy patients immediately before breakfast at 8.30 a.m. and by the other subjects 2–4 hours before. The experiment was started at 9 a.m. or later when the sonde had reached the middle or lower part of the small intestine. The position of the sonde was judged from the chart drawn by the antenna system.

A thin walled rubber balloon was introduced into the rectal ampulla and filled with water at 37°C until the patient felt urgency. The balloon had a neck 6 cm long and 1.2 cm in diameter, the axial length of the ovoid body of the balloon was 14.5 cm. Its inflated diameter undistended was 11 cm. The neck of the balloon was tied around a plastic catheter which was used for filling and pressure recording and also served as a support when the collapsed body of the balloon was to be inserted into the rectal ampulla. The filling was performed slowly in increments of about 25 ml with intervals for recording. As a rule it took about 10 minutes to reach the urgency threshold.

During urgency the pressure in the balloon rose steeply. The pressure can be considered equal to the rectal pressure since the capacity of the undistended balloon was always greater than the volume of water used. Several attacks of urgency were produced in each patient by varying the water volume. The

intraluminal anal pressure and the movements of the endoradiosonde were recorded continuously. In analysing the pressure curves the classification of Code Hightower & Morlock (2) was used.

Results

The course of events when the rectal balloon was filled slowly in a normal subject was as illustrated in figure 1. At a volume of between 225 and 500 ml the rectum suddenly contracted so that the balloon pressure rose from a reference value of 5–15 cm to 45–130 cm H₂O. Before this contraction the patient had experienced only a sensation of filling in the rectum. Simultaneously with the contraction, however, urgency and slight discomfort was felt, which was described as arising in the upper part of the abdomen as well as in the rectum. At the same time there was a burst of pressure waves from the small intestine consisting either of several type I waves or of a single type III wave. The patient was asked to avoid straining to prevent interfering pressure waves.

The filling of the balloon was always stopped at the onset of urgency, and in the control subjects the rectum then became adapted within 2 minutes, the balloon pressure falling to the reference value, the feeling of urgency disappearing and the pressure activity of the small intestine returning to normal. When relaxation was complete the normal rectum did not contract again even though the balloon volume was not changed for 5–8 minutes. To provoke a new attack of urgency it was necessary either to add

less than 2 minutes starting and ending at the reference line and accompanied by urgency. The individual contraction seemed not to differ from that for the controls where however it was not repeated. In none of the patients of this group was there any effect on the ileal pressure activity (Figure 4).

Two patients had undergone a *partial resection of the colon* for carcinoma and an end to end anastomosis had been performed in one of them between the proximal part of the transverse colon and the descending colon and in the other between the proximal part of the descending and the sigmoid colon. In the experiment these patients reacted as normal subjects in every respect.

Discussion

The recto ileal reflex described by Biriny & Jacobson in a preliminary communication (1) seems to have its trigger zone in the upper part of the rectum or at the rectosigmoidal junction. In patients who had had this part of the bowel resected no motility of the small intestine could be elicited by distension, the remaining adjacent parts.

The fact that only 2 of the 7 patients with truncal vagotomy had a normal reflex suggests that a vagal branch in the efferent pathway.

Using fluoroscopy Velho da Silva observed a recto-rectal reflex which implied a contraction of the cecum and ascending colon as a result of rectal distension (5). Connell Frankel

and Guttman found that distension of the rectum produced a pressure activity of the sigmoid colon which they suggested had the effect of retaining bowel contents in the colon while the rectum was being emptied (3). A study of the movements of the endoradio-sonde during attacks of urgency with and without synchronous pressure activity of the small intestine did not reveal any tendency for the recto ileal reflex to inhibit propulsion.

It seems justified for the subsequent discussion to assume that the recto ileal reflex is part of a more generalized activation of the bowel elicited by distension of the trigger zone. Since vagotomy can abolish the ileal response the activation would seem in some measure to depend upon vagal impulses. Other efferent nerves must however also be involved especially those to the transverse left and sigmoid colon. This could explain why patients that have undergone vagotomy and those with resection of the trigger zone both lack the recto ileal reflex while only the former assign part of the urgency to the upper abdomen.

The persistent relaxation of the rectum which normally follows within 2 minutes of a contraction provoked by distension implies adaptation to the distension and is essential for normal bowel habits. If the adaptation does not take place there will soon be an urgency to defecate irrespective of the time of day or of the number of previous bowel movements that day. Such a lack of adaptation to distension was found in all the cases of

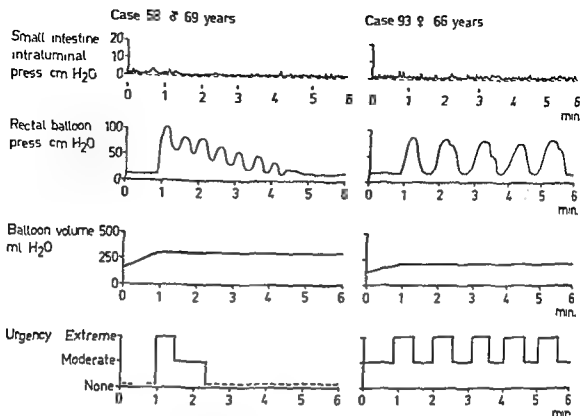


Fig 3

Fig 4

Fig 3 *Polpa resection with vagotomy* Prolonged period of rectal relaxation with pressure waves of decreasing amplitude. No pressure activity of the small intestine provoked by attack of urgency.

Fig 4 *Proximal rectum resected* Constant distension of bowel provoked a series of pressure waves. Complete relaxation between waves. No pressure activity of the small intestine corresponding to bowel contractions.

vagotomy The remaining patient showed a few rhythmic contractions of the rectum during the relaxation period, which however still terminated within the normal 2 minutes.

Four patients had undergone *resection of the upper part of the rectum* for a carcinoma and the anastomosis to the sigmoid was located 6–10 cm from the anus. The balloon volume necessary to produce contraction of the bowel was slightly less (175–215 ml) in these patients than in the controls. The contraction was accompa-

nied by a feeling of urgency, but this was felt only in the rectal region and not in the upper part of the abdomen as was the case in all other groups. Pressure activity of the small intestine synchronously with rectal contraction was not seen in this group. The bowel of these patients showed a remarkable activity as recorded from the balloon thus without any change of the balloon volume a seemingly unlimited series of pressure waves of fairly constant amplitude was obtained. The contractions occurred at intervals of

less than 2 minutes starting and ending at the reference line and accompanied by urgency. The individual contraction seemed not to differ from that for the controls where however it was not repeated. In none of the patients of this group was there any effect on the ileal pressure activity (Figure 4).

Two patients had undergone a *partial resection of the colon* for carcinoma and an end to end anastomosis had been performed in one of them between the proximal part of the transverse colon and the descending colon and in the other between the proximal part of the descending and the sigmoid colon. In the experiment these patients reacted as normal subjects in every respect.

Discussion

The recto ileal reflex described by Biriny & Jacobson in a preliminary communication (1) seems to have its trigger zone in the upper part of the rectum or at the rectosigmoidal junction. In patients who had had this part of the bowel resected no motility of the small intestine could be elicited by distending the remaining adjacent parts.

The fact that only 2 of the 7 patients with truncal vagotomy had a normal reflex suggests that a vagal branch is the efferent pathway.

Using fluoroscopy Velho da Silva observed a rectocaecal reflex which implied a contraction of the caecum and ascending colon as a result of rectal distension (6). Connell Frankel

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It seems justified for the subsequent discussion to assume that the recto ileal reflex is part of a more generalized activation of the bowel elicited by distension of the trigger zone. Since vagotomy can abolish the ileal response the activation would seem in some measure to depend upon vagal impulses. Other efferent nerves must however also be involved especially those to the transverse left and sigmoid colon. This could explain why patients that have undergone vagotomy and those with resection of the trigger zone both lack the recto ileal reflex while only the former assign part of the urgency to the upper abdomen.

The persistent relaxation of the rectum which normally follows within 2 minutes of a contraction provoked by distension implies adaptation to the distension and is essential for normal bowel habits. If the adaptation does not take place there will soon be an urgency to defecate irrespective of the time of day or of the number of previous bowel movements that day. Such a lack of adaptation to distension was found in all the cases of

functional diarrhea and in the patients who had undergone resection of the proximal part of the rectum and the rectosigmoidal junction. Furthermore, most of the vagotomized patients showed a slower adaptive relaxation of the rectum than the controls.

A comparison of these three groups indicates that the trigger zone in the rectum has two functions. It activates the gut through reflexes, which are partly vagal, and it is the origin of a slow inhibitory mechanism, which normally extinguishes the rectal contraction and the reflexes within 2 minutes.

In the cases of functional diarrhea there was activation but no inhibition. Distension of the rectum provoked an almost tonic contraction and a persistent recto ileal reflex. Urgency was felt in the upper abdomen and soon became unbearable. The question is whether the 'hyperirritability' of the gut in these patients is due to a local disturbance of the trigger zone or to a more generalized malfunction. In this connection it should be mentioned that the distension threshold for contraction was not obviously lower in the diarrhea patients than in the controls.

In patients where the trigger zone had been resected there was no evidence of activation or inhibition. The distension elicited an unlimited series of local pressure waves but no recto ileal reflex, and each wave was followed by complete relaxation. Urgency and discomfort were much less marked than in the diarrhea patients and were not felt in the upper abdomen. The resection patients did not

suffer from diarrhea but noticed a change in their bowel habits after the operation. They could no longer withstand a call to stool for more than a short period, and they usually had an evacuation after each meal.

In the vagotomized patients the activation was often partly eliminated, and then no recto ileal reflex was recorded, but urgency was still felt in the upper abdomen. The inhibition may also have been affected to some extent, since rectal adaptation was delayed. The clinical significance of these changes remains to be established.

Summary

The rectum of 14 healthy controls and 32 patients with various gastrointestinal disturbances was subjected to gradual distension by means of a water-filled balloon. Ileal motility was recorded with an endoradiosonde and rectal motility was recorded from the balloon.

Distension elicited a recto ileal reflex, except in 5 out of 7 patients that had undergone truncal vagotomy and in 4 with resection of the upper rectum.

Adaptation of the rectum to distension was normally complete within 2 minutes. It was absent in all 11 cases of functional diarrhea and in the 4 rectal resection patients. There was a marked difference between these two groups. The cases of functional diarrhea showed an almost tonic contraction of the rectum which was accompanied by a persistent recto ileal reflex.

during distension Urgency soon became unbearable

In the resection group the distension provoked a series of rhythmic contractions and relaxations but no recto ileal reflex Urgency was much better tolerated and there was no diarrhea

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The three patients

By GUNNAR BJÖRCK

During the years 1950—1958 I had the privilege to work under and with Jan Waldenstrom in Malmö. In this period, carcinoids, heart surgery and the epidemiology of atherosclerosis all presented challenges to the cardiologists of his medical department. One of the areas, into which we had to expand was the automatic processing of information from many thousands of hospital records concerning patients with rheumatic fever, valvular heart disease and myocardial infarction. Experiences from our work in Malmö were fundamental to us when P Hall and I during the 1960's were forced into the creation and development of a center for automatic processing of medical records in my department of medicine at Serafimerhälsarettet Stockholm.

The handling of hospital records by data technique is not only, and not mainly, a technical problem. The challenge is primarily a medical one to the physician who has to define what the significance of the record is in his method of dealing with the patient. The problem thus, is one of describing in terms of operational

analysis, how the physician works and at the same time to identify the structure, and substructures, of a hospital record. For not only must the record make sense to the doctor, it must at the same time be construed as to make sense to a data machine. Such endeavours may appear somewhat painful to the clinician for whom so much of this has been imbibed into his central nervous system as to have assumed the quality of intuition rather than technique.

I shall not venture into philosophy but I think it is fair to state that there are always *two* patients: the *real* one and the *image* of him in the physician's mind (Fig. 1). But in hospital medicine there are always at least *two imaginary* patients for every real one: the image in the doctor's mind and the *patient-on record*. The patient on record is at times more informed and more informative than the real one (although occasionally he will tell us as much about the author of the record as of the patient). In most cases the patient on record and the real patient differ in composition (Fig. 2) in some respects the record

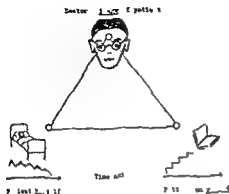


Fig 1

goes deeper under the surface as is true of data on earlier disease which the patient himself may have forgotten about or of laboratory data — in other respects the patient may be hiding important information below the surface making it unaccessible for the record which I believe is particularly true of psychological data

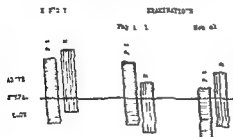


Fig 2

In his work the physician operates with all the three versions of the patient the real patient the patient on record and the image in the physician's mind which consists of the other two mixed and distorted by the physician's own temperament

Fig 3

PHYSICIAN

PATIENT

RECORD

Physician compares

PATIENT \neq IMAGEPATIENT \neq RECORDRECORD \neq IMAGE

A hospitalized patient proceeds along a time axis and the physician tries to keep his image of the patient up to date by comparing impressions from the real patient with information contained in the record (Fig 3). There is to day an appreciable tendency among junior doctors to study the record closer than the patient. This is a problem which merits a paper by itself.

A few words might be added concerning the production of the medical record. In a quantitatively limited medical practice the physician might even do without records relying only on his own memory. However in our country a law has recently made record writing obligatory for all physicians who treat patients. With greater patient loads and a more sophisticated technology the record writing may develop along various lines as seen in fig 4. It is interesting to note that in the case of the medical history the sequence may be radically changed through active participation by the patient at least to a certain extent (selfadministered questionnaire). It is also worth remembering that many pieces of information such as laboratory data consultant opinions etc may be entered in the record without

Fig 4

PHYSICIAN

Information → own memory store

Information → own memory store → own handwritten record

Information → own memory store → secretary → typewritten record

Information → own memory store → dictaphone → secretary → typewritten record

Information → own memory store → dictaphone → secretary → punch tape

→ magnetic memory → automatically typewritten record

SELF ADMINISTERED QUESTIONNAIRE

Information → secretary → punch tape → magnetic memory → automatically

typewritten record → physician → own memory store

passing first through the attending physician

Now, how does the doctor work with the real patient, the patient on

record and the image of the patient?

Figure 5 represents an attempt to indicate, briefly and inadequately, the various levels at which activities oc

Fig 5

	DOCTOR—PATIENT	DOCTOR'S IMAGE	RECORD
1	Take history		Notes
	Explain calm	Concept of patient	
2	Examine		Notes
	Alleviate pain		Assort notes
3			Write record
4		"Case"	
5		Interpret	
6		Analyse possibilities	
7		Conclude diagnosis (if insufficient evidence Repeat)	Record
8	Further examinations		Add new data
9		Final diagnosis	Record
10	Inform patient		Record
11		Decide on action	Record treatment
12	Treat		Record effects
13	Observe effects		
14		Evaluate treatment	Summarize
15	Inform advise		Store
16		Remember — or forget	

cur I believe it demonstrates that the record is an indispensable instrument by means of which the running medical decision making is made possible at the same time as the decisions and the basis upon which they are founded are codified

The fact that disease in and care of a patient is a dynamic process and not a static situation represents one of the big difficulties in collecting and recording of medical data. Most information on a patient is obtained point wise and only rarely continually — with the exception of continuous monitoring of cardiovascular and similar parameters in some intensive care units. The more careful or even continuous recordings of patient data along a time axis are the more bulky becomes the collected material the more difficult to grasp for the physician and the more imperative becomes the need of concentration and the boiling down of the material to essentials. In as much as an increasing percentage of our patients are older people often with a loaded medical history with many more recorded examinations and episodes of disease than formerly with more treatment and medication and — perhaps — with a more extensive interplay of medical psychological and social factors than hitherto recognized the medical record of today is doomed to become a more comprehensive but less digestible document than in earlier times. Certainly this is one of the big problems before us. The data machine can reduce the storage space to a few centimeters on a magnetic tape but it

cannot by itself condense the material into a truly representative and useful summary

We know from the experience of others and ourselves that the data machine to day can accumulate store and reproduce the alpha numerical information contained in the conventional medical record. This mere fact does not tell us whether a data machine produced medical record is better or worse than a conventional one. But it does imply that the problem of record storage is solved and that statistical analyses of various kinds are greatly facilitated. It will also facilitate the production of check off lists for future follow up studies of patient materials.

However it is but natural that we are asking ourselves what possibilities with regard to the record proper are inherent in present data techniques. What ways and means do we have to increase the amount of useful information in the individual patient and to make this information more precise? We know that there is much to be gained by standardization of questions and procedures — but we do not know what might be lost by the rigidity imposed by such methods (fig 6).

Above all we want to know whether the data technique will eventually offer us in addition to information also interpretation diagnostic alternatives and recommendations for supplementary examinations as well as for the therapy in case of established diagnosis or a preferential sequence of therapeutic trials in case of undecided diag-

Fig 4

PHYSICIAN

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Information → own memory store → dictaphone → secretary → typewritten record

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SELF ADMINISTERED QUESTIONNAIRE

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6		Analyse possibilities	
7		Conclude diagnosis (If insufficient evidence Repeat)	Record
8	Further examinations		Add new data
9		Final diagnosis	Record
10	Inform patient		
11		Decide on action	Record
12	Treat		Record treatment
13	Observe effects		Record effects
14		Evaluate treatment	
15	Inform advise		Summarize
16		Remember — or forget	Store

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AB Kabi	AB Meda
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In the same sense of congratulation a great number of Swedish colleagues have paid homage to Jan Waldenström on his birthday by sponsoring this volume. Their names will be found in a special address of greeting which will be handed over to the celebrator on April 17 1966.

Fig 6

INFORMATION

Accumulated factual data

(history, examination laboratory)

INTERPRETATION

Analysis of possible diagnoses establish
priority order

RECOMMENDATIONS

1 DIAGNOSTIC→supplementary examinations

2 THERAPEUTIC

a) Diagnosis established —
treatment of choiceb) Diagnosis not established —
preferential sequence of trials

nosis This might appear to be easily within reach, but people often forget that clinical medicine is not an absolute science, many of its units and measures are arbitrary and subjective, with equally arbitrary and subjective dividing lines between normal and abnormal along a continuity, and — in addition to that — patients often have more than one disease at the same time. To which one of an acute purulent creatitis an abstinence delirium or a

bronchopneumonia occurring simultaneously in a patient should we ascribe an elevated body temperature? It might also be of interest to investigate, whether the prevailing diagnostic concepts, as they have emerged through centuries, still with an easily discernible foundation in gross microscopic pathology, might profitably be moulded upon other conceptual patterns

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ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 446

STUDIES ON HUMAN SERUM VERY-LOW-DENSITY-LIPOPROTEINS

BY
ANDERS GUSTAFSON

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LP the method of determining and expressing protein content has often been a matter of discussion. In the present investigation a comparative study was performed on washed and unwashed representative subfractions of the VLD-LP and fresh standards, human albumin. The amount of protein was generally determined by the method of Lowry et al. (53). As this method measures only the tyrosine content of the protein it was compared with the biuret method. The concentration of the protein was also determined as 6.25 times the Kjeldahl nitrogen. Good agreement was obtained between the three methods, suggesting that the tyrosine content of the VLD LP proteins and the human albumin was the same. Therefore, no corrections have been made.

In Table A the composition was recalculated as mole lipids and amino acid residues per g molecular weight of LP. Composition data and LP molecular

weights were obtained from Paper I. The data used for molecular weights of lipid components and amino acid residues are given in the table.

For added convenience in the comparison of the molar composition of different subfractions the lipid mole ratio has also been expressed per 100 amino acid residues (Table B). From data in this table certain similarities in the lipid protein composition are apparent. The chylomicron fractions, fractions A and B, agree well in molar as well as in per cent composition (Table IV). Fractions D, E and β -LP (data of Oncley, 1963) (50) reveal, when calculated by their mole lipid ratios, an interesting relationship. The decrease in relative triglyceride content concomitant with increase in LP density is, of course, to be expected.

The cholesterol ester content showed the greatest variation within an individual fraction. It seems established that

Table A. Lipid and protein composition of five VLD lipoprotein fractions and the β -lipoproteins. Data expressed as mole lipids and amino acid residues per g mol wt of lipoproteins.

Lipoprotein Fraction	Mol Wt ($\times 10^6$)	Mole Lipids and Amino Acid Residues per g Mol Wt. of Lipoprotein				
		FC	CE	PL	TG	PR
Fraction A	> 5000	26×10^4	31×10^4	27×10^4	497×10^4	47×10^4
Fraction B	233	18×10^4	26×10^4	20×10^4	212×10^4	36×10^4
Fraction C	27	27×10^4	45×10^4	40×10^4	206×10^4	122×10^4
Fraction D	15	16×10^4	31×10^4	27×10^4	100×10^4	119×10^4
Fraction E	8	670	1240	1020	3820	6240
β -LP ^a)	2.3	470	1300	670	280	4100

FC = Free cholesterol (Mol wt. 386) CE = Cholesterol ester (Cholesteryl stearate Mol wt. 654)

PL = Phospholipid (Lecithin Mol wt. 775) TG = Triglyceride (Tristearate Mol wt. 893) and

PR = Amino acid residues (Avg. Mol wt. 117)

^a) From data of Oncley 1963 (50)

Methods

CHAPTER I

Isolation and characterization of VLD-lipoprotein fractions (Paper I)

Incompleteness in techniques and data in earlier investigations on the VLD-LP prompted efforts to find new ways for their isolation and fractionation. Ultracentrifugal separation based on differences in density used in the case of higher density LP is not easily applicable to LP of low density, $D < 1.006$. Density gradient techniques have been applied in the subfractionation of serum LP (50), in the case of chylomicrons and VLD-LP in combination with a specially designed swinging-bucket ultracentrifuge (51).

In the present study, preparative ultracentrifugation was employed in an angle head centrifuge applying increasing centrifugal force and time (expressed as the product $g \times \text{min}$) on each of several successive runs. At the end of each isolation step the floated VLD-LP were recovered from the top ml of the centrifuge tube. Theoretically this technique may give any number of narrow VLD-LP subfractions.

Inconsistency in density of human sera made it necessary in the isolation steps to layer over the original serum sample and subsequent infranatants with a column of buffer solution, $D 1.006$. The procedure of overlaying has been used previously in the isolation of chylomicron

fractions (52) to prevent a too heavy contamination of serum proteins. The present study demonstrated that layering was helpful in diminishing contamination of adjacent VLD LP and serum proteins in the isolation of any VLD-LP subfraction. Repeated ultracentrifugations, "washings" of the re-suspended LP were always necessary, however. These washings were performed at the same gravitational force as the preceding isolation step. The goal of purification was to obtain a fraction that had the same protein composition after two consecutive washings and that was homogeneous on electrophoresis.

Based on these experiences a standardized procedure was outlined for the isolation and purification of five arbitrarily chosen VLD-LP subfractions A, B, C, D and E at centrifugal force 0.1×10^6 , 0.6×10^6 , 4.8×10^6 , 12.6×10^6 , and 139×10^6 g min, respectively. As much as 45-50% of the original lipid content of a subfraction was lost by the washings. Subjects with hyperglycemia which had been characterized previously were therefore used as donors to allow sufficient amounts of LP to be recovered and studied.

In the chemical characterization of

Introduction

It is now accepted knowledge that the water insoluble lipids cholesterol, cholesterol ester, phospholipid, triglyceride and fatty acids present in serum circulate bound to specific proteins forming *macromolecular complexes, lipoproteins (LP)*

The earliest evidence for the existence of lipid protein complexes in serum can be traced back to Nerking in 1901 (1) Lipid phosphorus (2) and cholesterol ester (3) were found in precipitated serum globulin preparations suggesting to these workers that serum lipids were transported by serum globulins Furthermore studies on free electrophoresis by Bennhold (4) Mellander (5), and Blix Tiselius and Svensson (6) in 1941 showed the major portion of the serum lipids within the α and β globulin fractions

In 1935 Mc Farlane (7) discovered the χ protein and Pedersen (8-9) in the years 1938—1947 revealed and confirmed its LP character Gofman (10) in 1949 stimulated by the observations of Pedersen presented a method for the quantitation by analytical ultracentrifugation of low density LP $D < 1.063$ g/cc In addition the Gofman group (10) introduced the Sf nomenclature defining these low density LP by their flotation characteristics (in the ultracentrifugal field) in a sodium chloride

solution of $D 1.063$ g/cc and at temperature $25-27^{\circ}\text{C}$ *)

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BY
ANDERS GUSTAFSON

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The present thesis is based on the following publications

- I Studies of the composition and structure of serum lipoproteins
Isolation, purification, and characterization of very low density lipoproteins of human serum
(Together with P Alaupovic and R H Furman)
Biochemistry 4 596, 1965
- II A new method for partial delipidization of serum lipoproteins
Biochim Biophys Acta 84 223, 1964
- III New method for partial delipidization of serum lipoproteins
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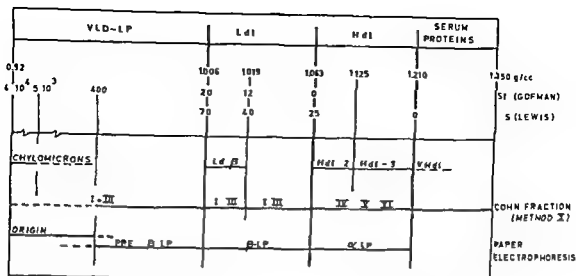


Figure A The serum LP spectrum LP designation by ultracentrifugal and electrophoretic methods and their relationship to hydrated density, Sf value (according to Gofman) - S value (according to Lewis) and to fractions of the isolation method by Cohn et al, Method X (13) The figure is based mainly on data collected by Cornwell and Kruger (20)

major somewhat overlapping LP classes have been identified within D 0.92—1.21 (Figure A), viz the chylomicrons, the very-low-density (VLD) LP, the low-density LP (Ldl) and the high-density LP (Hdl). Each of the four classes has been assumed to have its characteristic protein moieties, metabolism and function.

The chylomicrons (24), primary particles (25) or alimentary particles (26) are large, light-scattering fat particles of alimentary origin, that normally appear in serum after a meal containing fat. The problem of separating the chylomicrons from the VLD-LP *per se* has not yet been completely solved. The LP of this heterogeneous class have in common the characteristic that they float in the ultracentrifugal field at D 1.006. The major portion of these triglyceride-rich and protein-low LP migrate electrophoretically with the α_2 -globulins, or as

a "pre- β fraction, α_2 - or pre- β -lipoproteins (Figure A). Metabolically these VLD-LP seem to be involved in the transport of endogenous glycerides (27).

The low-density LP (Ldl), also named the β -LP from their electrophoretic mobility, is quantitatively the largest LP class in man and also the major cholesterol-bearing LP. These LP are possibly related metabolically to the VLD-LP (28).

The protein and phospholipid-rich high-density LP (Hdl) or α -LP float at ultracentrifugation between D 1.063—1.21 (Figure A). The high-density LP can be subfractionated into Hdl-2 and Hdl-3 (29) having different lipid-protein ratios and physical characteristics but an identical protein moiety (30) and possibly identical physiological function. In the serum residue at D 1.21 i.e. the infranantant at solvent density 1.21 g/cc a phospholipid-protein complex, called

the very high density LP (VHdl) has been identified (31, 32) containing a protein moiety identical to that of the α LP (33, 34). With the LP classes is often also included the free fatty acid (FFA)-albumin complex in the infranatant at D 1 21.

The terms hypercholesterolemia, hyperlipemia and hyperlipidemia have been used to characterize disease entities with an elevation in one or more lipid components of the serum. With the introduction of LP fractionation methods it has been apparent that the serum lipid changes reflect variations in amount of LP rather than changes in the composition of these LP. It has therefore been found more appropriate to evaluate the serum lipid variations in terms of LP changes.

The characteristic feature of essential hyperlipemia is an abnormal rise in serum triglyceride, in turn due to an increase in triglyceride-rich VLD LP. Several clinical types of hyperglycemia occur (Chapter III).

The term essential hypercholesterolemia indicates a higher than normal amount of cholesterol in the serum, reflecting an increase in cholesterol rich β LP (hyper β lipoproteinemia). A disease entity with a combined hyper lipoproteinemia elevated VLD LP and β LP has been observed (see Chapter III). So far no cases of essential hyper- α lipoproteinemia have been reported.

Congenital deficiency of a lipoprotein class α lipoproteinemia has recently been described for both β LP and α LP. Lack

of β LP, α - β -lipoproteinemia, Bassen-Kornzweig syndrome (35, 36) has clinically a malignant course with among other signs a defective exogenous fat transport system. The α - α lipoproteinemia, the Tangier disease (37, 38), on the other hand causes only a mild reticuloendotheliosis and has an apparently normal chylomicron production.

The research on serum lipids and LP was stimulated tremendously when in the early fifties it was suggested (39) that there might well be a relationship between increased levels of serum lipids and early coronary heart disease. Different lipids and lipoprotein classes have been accused of atherogenic properties during the successive development in the knowledge of lipids. Serum cholesterol (40), serum triglyceride (41, 42, 43), serum cholesterol and triglyceride (44), β LP (Sf 10 20) (39), VLD LP (Sf 20 100) (45, 46) and combinations of these parameters (atherogenic index) (47) all have been suggested predictors of subclinical arterial disease.

Even if now a multifactorial cause for atherosclerosis has been established (48) the important participation of serum lipids and lipoproteins in this process can hardly be denied.

This study was initiated by the finding of Furman et al., (49) of a high density LP being released from VLD LP by sonic forces. The purpose of the present investigation was to characterize the complex class of VLD LP, including the chylomicrons and ultimately their protein moieties.

Methods

CHAPTER I

Isolation and characterization of VLD-lipoprotein fractions (Paper I)

Incompleteness in techniques and data in earlier investigations on the VLD-LP prompted efforts to find new ways for their isolation and fractionation. Ultracentrifugal separation based on differences in density used in the case of higher density LP is not easily applicable to LP of low density, $D < 1.006$. Density gradient techniques have been applied in the subfractionation of serum LP (50), in the case of chylomicrons and VLD-LP in combination with a specially designed swinging-bucket ultracentrifuge (51).

In the present study, preparative ultracentrifugation was employed in an angle head centrifuge applying increasing centrifugal force and time (expressed as the product $g \times \text{min}$) on each of several successive runs. At the end of each isolation step the floated VLD-LP were recovered from the top ml of the centrifuge tube. Theoretically, this technique may give any number of narrow VLD-LP subfractions.

Inconsistency in density of human sera made it necessary in the isolation steps to layer over the original serum sample and subsequent infranatants with a column of buffer solution, $D 1.006$. The procedure of overlaying has been used previously in the isolation of chylomicron fractions (52) to prevent a too

heavy contamination of serum proteins. The present study demonstrated that layering was helpful in diminishing contamination of adjacent VLD-LP and serum proteins in the isolation of any VLD-LP subfraction. Repeated ultracentrifugations, "washings" of the resuspended LP were always necessary, however. These washings were performed at the same gravitational force as the preceding isolation step. The goal of purification was to obtain a fraction that had the same protein composition after two consecutive washings and that was homogeneous on electrophoresis.

Based on these experiences a standardized procedure was outlined for the isolation and purification of five arbitrarily chosen VLD-LP subfractions A, B, C, D, and E at centrifugal force 0.1×10^6 , 0.6×10^6 , 4.8×10^6 , 12×10^6 , and 139×10^6 g min, respectively. As much as 45-50% of the original lipid content of a subfraction was lost by the washings. Subjects with hyperglyceridemia, which had been characterized previously, were therefore used as donors to allow sufficient amounts of LP to be recovered and studied.

In the chemical characterization of

LP the method of determining and expressing protein content has often been a matter of discussion. In the present investigation a comparative study was performed on washed and unwashed representative subfractions of the VLD LP and fresh standards human albumin. The amount of protein was generally determined by the method of Lowry et al. (53). As this method measures only the tyrosine content of the protein it was compared with the biuret method. The concentration of the protein was also determined as 6.25 times the Kjeldahl nitrogen. Good agreement was obtained between the three methods suggesting that the tyrosine content of the VLD LP proteins and the human albumin was the same. Therefore, no corrections have been made.

In Table A the composition was recalculated as mole lipids and amino acid residues per g molecular weight of LP. Composition data and LP molecular

weights were obtained from Paper I. The data used for molecular weights of lipid components and amino acid residues are given in the table.

For added convenience in the comparison of the molar composition of different subfractions, the lipid mole ratio has also been expressed per 100 amino acid residues (Table B). From data in this table certain similarities in the lipid protein composition are apparent. The 'chylomicron fractions', fractions A and B, agree well in molar as well as in per cent composition (Table IV). Fractions D, E and β LP (data of Oncley 1963) (50) reveal, when calculated by their mole lipid ratios, an interesting relationship. The decrease in relative triglyceride content concomitant with increase in LP density is, of course, to be expected.

The cholesterol ester content showed the greatest variation within an individual fraction. It seems established that

Table A. Lipid and protein composition of five β -LDLipoprotein fractions and the β -lipoprotein. Data expressed as mole lipids and amino acid residues per g molecular weight of lipoprotein.

Lipoprotein Fraction	Mole Lipids and Amino Acid Residues per g Mol Wt. of Lipoprotein					
	Mol Wt. ($\times 10^4$)	FC	CE	PL	TG	PR
Fraction A	> 5000	26×10^4	31×10^4	27×10^4	497×10^4	47×10^4
Fraction B	233	18×10^4	26×10^4	20×10^4	212×10^4	36×10^4
Fraction C	27	27×10^4	45×10^4	40×10^4	206×10^4	122×10^4
Fraction D	15	16×10^4	31×10^4	27×10^4	100×10^4	119×10^4
Fraction E	6	670	1240	1070	3820	6240
β LP ¹⁾	2.3	40	1300	670	280	4100

FC = Free cholesterol (Mol wt. 386) CE = Cholesterol ester (Cholesteryl stearate Mol wt. 654)

PL = Phospholipids (Lecithin Mol wt. 775) TG = Triglyceride (Tristearate Mol wt. 873) and

PR = Amino acid residues (Avg. Mol wt. 117)

From data of Oncley 1963 (50)

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micrographs (28), which also showed a spherical although somewhat flattened shape

The electrophoretic mobility of VLD-LP on agar gel (Figure 2) appear better explained in the light of the new knowledge of their protein moieties (Chapter V) The presence of β LP protein, and the known interference of β -LP with agar at different levels of protein concentration (54), may explain both the trailing of fraction C and the slight difference in mobility between fractions D and E (Figure 2)

It is apparent from the present study that LP of different size and chemical composition can be obtained by varying the speed and the duration of the ultracentrifugation Discrepancies in chemical and physical data in chylomicrons and VLD LP fractions obtained in different laboratories may be due to the lack of a standardized technique for the separation of these LP By the use of the experimentally obtained graph (Figure 5) relating Sf value of the LP maximum peak and ultracentrifugal force (g min) used for the isolation of the LP, such a standardization could be achieved

CHAPTER II

Partial delipidization (Papers II and III)

Complete delipidization of β -LP (55) and VLD LP (56) failed to give water soluble residues A nearly lipid free protein moiety was however obtained in water soluble form from α LP (57)

Methods for partial delipidization have thus far utilized a biphasic system of lipid solvents and water Using diethyl ether isolation of reproducible residues could not be obtained and there was a low yield of water soluble material particularly from VLD LP Since these were of primary interest in the present study a new method for partial delipidization was developed

Lyophilization of serum LP caused irreversible changes in the protein moiety (58, 59) However, it was known that the protein denaturation on freeze-drying may be prevented by the presence of

glucose, glycerol, sucrose (58) and insoluble starch (60) This effect can possibly be explained by the hydroxyl groups of these compounds substituting for protein hydration water

Furthermore, the neutral lipid extraction was accelerated by the presence of starch, exposing the lyophilized LP to a large contact area with the lipid solvent (61) From ether delipidization experiments it was noted that the vulnerability of the LP protein moieties was due to the degree of phospholipid extraction as well as to the extent of dehydration By using a non polar lipid solvent, *n* heptane, less phospholipid was extracted and the yield of soluble phospholipid protein residues was improved

The lipid extraction was interrupted when all neutral lipids had been

repeated ultracentrifugations and resuspensions, besides removing contaminants, also cause some loss of cholesterol ester. This is the most likely explanation for the observation that the content of cholesterol ester was lower than expected in fractions D and E (Table B).

From the experimentally determined Sf^0 value and the estimated value for LP density, the approximate molecular weight (Figure B) of the average LP of the five subfractions was calculated. These molecular weights agree well with those obtained from the electron

Table B Lipid and protein composition of five VDL lipoprotein fractions and the β -lipoproteins. Data expressed as mole lipids per 100 amino residues

Lipoprotein Fraction	LP Density (g/cc)	Mole Lipids per 100 Amino Acid Residues				
		FC	CE	PL	TG	PR
Fraction A	0.928	55	65	58	1060	100
Fraction B	0.936	49	73	55	590	100
Fraction C	0.957	22	37	33	170	100
Fraction D	0.977	13	26	23	84	100
Fraction E	0.989	11	20	16	61	100
β LP ¹⁾	1.03	11	32	16	7	100

See Table A for abbreviations

¹⁾ Composition data from Oncley 1963 (50)

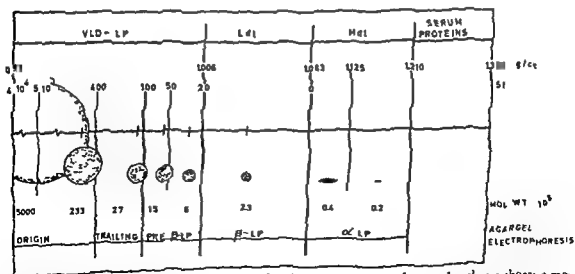


Figure B The serum LP spectrum. Relationship between LP molecular weight, electrophoretic mobility on agar gel and the LP characteristics of hydrated density and Sf value. Data from the present study, except molecular weights of α LP (87) and β LP (50)

micrographs (28) which also showed a spherical although somewhat flattened shape

The electrophoretic mobility of VLD-LP on agar gel (Figure 2) appear better explained in the light of the new knowledge of their protein moieties (Chapter V). The presence of β LP protein, and the known interference of β LP with agar at different levels of protein concentration (54), may explain both the trailing of fraction C and the slight difference in mobility between fractions D and E (Figure 2)

It is apparent from the present study that LP of different size and chemical composition can be obtained by varying the speed and the duration of the ultracentrifugation. Discrepancies in chemical and physical data in chylomicrons and VLD-LP fractions obtained in different laboratories may be due to the lack of a standardized technique for the separation of these LP. By the use of the experimentally obtained graph (Figure 5) relating S_f value of the LP maximum peak and ultracentrifugal force ($g \cdot min$) used for the isolation of the LP, such a standardization could be achieved

CHAPTER II

Partial delipidization (Papers II and III)

Complete delipidization of β LP (55) and VLD LP (56) failed to give water soluble residues. A nearly lipid-free protein moiety was, however, obtained in water soluble form from α LP (57)

Methods for partial delipidization have thus far utilized a biphasic system of lipid solvents and water. Using diethyl ether, isolation of reproducible residues could not be obtained and there was a low yield of water soluble material, particularly from VLD LP. Since these were of primary interest in the present study, a new method for partial delipidization was developed.

Lyophilization of serum LP caused irreversible changes in the protein moiety (58-59). However it was known that the protein denaturation on freeze-drying may be prevented by the presence of

glucose, glycerol, sucrose (58) and in soluble starch (60). This effect can possibly be explained by the hydroxyl groups of these compounds substituting for protein hydration water.

Furthermore, the neutral lipid extraction was accelerated by the presence of starch, exposing the lyophilized LP to a large contact area with the lipid solvent (61). From ether delipidization experiments it was noted that the vulnerability of the LP protein moieties was due to the degree of phospholipid extraction as well as to the extent of dehydration. By using a non polar lipid solvent, n heptane less phospholipid was extracted and the yield of soluble phospholipid protein residues was improved.

The lipid extraction was interrupted when all neutral lipids had been

removed. This was determined by thin-layer chromatography of the consecutive lipid extracts (Figure 1). At the end of lipid extraction a certain amount of phospholipid, characteristic for each LP fraction, was left unextracted in the residue of all serum LP fractions (Table 1).

The composition of the phospholipid-protein residues suggests, in agreement with earlier studies (62, 63), that in all serum LP, the phospholipid component is bound to the protein moiety by stronger bonds than the neutral lipids, and also that the phospholipid in VLD and β -LP serves as a stabilizing agent for the protein moiety.

The thin-layer chromatograms of the lipid extracts revealed that of the neutral lipids, the free cholesterol was more tightly bound in the β -LP than the cholesterol ester and triglyceride. The evidence for a loosely bound cholesterol

ester is in agreement with earlier findings (Paper I) of a cholesterol ester loss by repeated ultracentrifugations. In α -LP apparently the lipid binding properties are different (64). In this LP, cholesterol ester is more tightly bound than free cholesterol, as judged by the sequence in the lipid removal.

The carotenoids were studied by following the presence and intensity of their characteristic yellow color within LP and degradation products. Their extractability showed an interesting similarity to that of the phospholipid, as might be expected from earlier studies (65). Only little was extracted by heptane and the major portion apparently remained with the phospholipid-protein residue. VLD and β LP contained carotenoids, while α -LP and chylomicrons did not show any visible amount.

Lipid moieties of the VLD-lipoproteins

CHAPTER III

VLD-lipoprotein composition and distribution in various hyperlipemic states

With the isolation of VLD LP subfractions in sera from various hyperglyceridemic donors it soon appeared that the distribution of total lipids in the five subfractions varied according to certain patterns. In this chapter, a limited study on the total lipid (and LP) distribution among VLD-LP fractions in three clinical types of hyperlipemia, will be described.

A brief review in the present knowledge of clinical typing of hyperlipemias will be given first.

Fat induced (66), *fat inducible* (67) hyperlipemia or according to the recent typing of Fredrickson and Lees (67), *hyperlipemia type I*, is, in its severe familial form, quite rare. It is characterized by the development of marked hyperlipemia, hyperglyceridemia even on a normal fat diet. A low level of plasma post heparin lipolytic activity (PHLA) (67) and heparin unresponsiveness (68) is felt to indicate a low activity of LP lipase (in the tissue) (67) or a defective LP lipase system (68). Interestingly enough so far there have been no reports demonstrating an accelerated atherosclerosis among subjects with this lipid disorder, although serum

triglyceride values often reach very high levels (Table C, subject H H).

Carbohydrate-induced (66), *carbohydrate-inducible* (67), *dietary carbohydrate accentuated* (69) hyperlipemia, *type IV* according to Fredrickson and Lees (67), has sensitivity of serum triglyceride level to dietary carbohydrate intake as a characteristic feature. These subjects may also show other evidence of a defective glucose metabolism, abnormal intravenous glucose tolerance test (49, 66) abnormal tolbutamide test or a low serum insulin like activity (66) and often a family history of diabetes (49, 70). This type has a serum lipid pattern that has been found to indicate special proneness to coronary heart disease in young men (67).

Other types of primary hyperlipemia hyperglyceridemia occur. In one other type suggested by Fredrickson and Lees (67), *the type V*, or *the fat and carbohydrate-inducible* form of hyperlipemia, the serum triglyceride levels were affected by both fat and carbohydrate intake. Simplified this type may combine the features of type I and type IV.

Clinical data and type of hyperlipemia of eight hyperglyceridemic subjects in-

removed. This was determined by thin-layer chromatography of the consecutive lipid extracts (Figure 1). At the end of lipid extraction a certain amount of phospholipid, characteristic for each LP fraction, was left unextracted in the residue of all serum LP fractions (Table 1).

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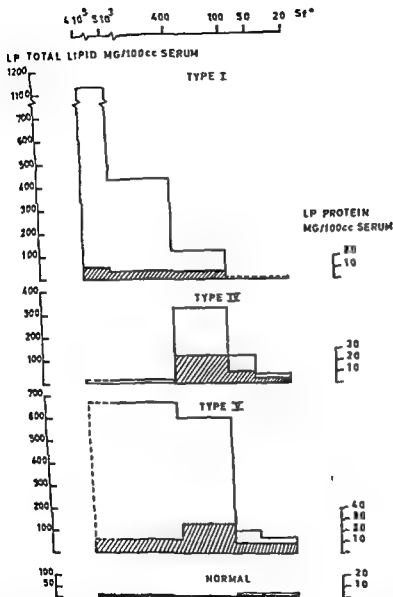


Figure C. Total lipid and LP protein distribution among VLD LP subfractions in three dietary types of hyperlipemia and in the normal subject. Distribution estimated from washed preparations, and expressed in mg per 100 cc serum.

cluded in this limited study are given in Table C. These subjects were also used as donors in the work described in Paper I and, with the addition of pooled sera from five more subjects, in the investi-

gations on the VLD-LP protein moieties, (Papers IV-V)

The subfractionation technique described in Paper I was utilized in this study. Total lipid and, in washed prepa-

Table C *Clinical and laboratory data and type of hyperlipemia in eight hyperglyceridemic blood donors*

Sex	Serum Lipids		Clinical History			Physical Signs		Tests	
	Cholesterol (mg/100 cc)	Triglyceride (mg/100 cc)	Kinship	Alcohol Consumption	Evidence of Vascular Disease	Xanthomas	Hepatosplenomegaly	I v Heparin**)	I v Glucose Tolerance Test**)
F	172	1645	Siblings	—	—	—	+	Unresponsive	Normal
F	140	685		—	—	—	+	Unresponsive	Normal
M	238	3488		—	—	—	+	Unresponsive	Normal
M	546	1131		—	Intermittent claudication	Tuberous	+	Normal	Impaired
M	314	398	Brothers	Moderate	—	Tuberous	+	Normal	?
M	310	869		—	Myocardial infarct at 47	—	—	Normal	Impaired
F	470	797	—	Moderate	Myocardial infarct at 54	—	—	Normal	Normal
M	334	948	—	Excessive	Cerebral vascular accident at 65	—	—	Normal	Mild diabetes

lung to Fredrickson and Lees 1963 (67)

None of these data the author is indebted to Drs O Brusco C W Robinson Jr and R P Howard

Table D *Per cent total lipid distribution among VLD lipoprotein fractions Comparison between unwashed and washed preparations*

Type of Hyperlipemia	Subject	Fraction Preparation	Per Cent Total Lipid Distribution					
			SF 40 000	SF 5 000	SF 400	SF 100	SF 50	SF 20
Type IV	O C	Unwashed	—	16.2	—	63.6	14.4	5.8
	O C	Washed	—	10.1	—	68.4	17.0	4.5

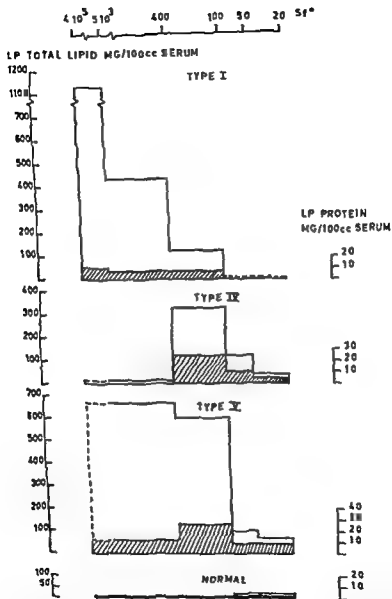


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F	172	1645	Siblings	—	—	—	+	Unresponsive	Normal	
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M	238	3488		—	—	—	+	Unresponsive	Normal	
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			Sf 40 000	Sf 5 000	Sf 400	Sf 100	Sf 50	Sf 20
Type IV	O. C.	Unwashed	—	16.2	—	63.6	14.4	5.8
	O. C.	Washed	—	10.1	—	68.4	17.0	4.5

VLP LP distribution shown on analytical ultracentrifugation by Gofman et al (71)

In comparing composition data it seems that the VLD LP, Sf 20—100 in type IV showed a higher cholesterol ester content (Table G) As was stressed before (Chapter I) the cholesterol ester content is however the most variable of the components of any VLD LP fraction The results of Lindgren et al (28) indicated a similar difference in cholesterol ester in normo- and hyperglyceridemic sera

In two brothers (G T and R T) with clinical data suggesting type V hyperlipemia the total lipid distribution showed a combination of the patterns

of type I and type IV Both VLD LP of Sf 100—400 and chylomicrons, Sf > 400 showed increased amounts of total lipid (Figure C Table E) This is in agreement with findings of Fredrickson and Lees from fat electrophoresis (67) Interestingly however the lipid particles recovered in Sf > 400 in type V in the present study showed a composition and appearance that differed from that of typical chylomicrons (Table H) Similarly Jobst and Schettler (72) observed a difference in composition between normal chylomicrons and those isolated from certain hyperlipemic sera

Table F Percent total lipid distribution among VLD lipoproteins of type I and type IV Comparison between experimental subjects with type I and type IV

Type of Hyperlipemia	Subject	Percent Total Lipid Distribution					
		Sf 40 000	Sf 5 000	Sf 400	Sf 100	Sf 50	Sf 20
Type I	H H	73.6	18.0	8.4	—	—	—
	H H	67.2	25.4	7.4	—	—	—
Type IV	O W	0.9	4.8	45.9	48.4	—	—
	O W	—	3.0	65.6	23.4	8.0	—

Table C Lipid and protein composition of VLD lipoproteins Sf 20—100 in hyperlipemic and normal subjects Data expressed as mg lipids per 100 mg amino acid residues

Type of Hyperlipemia	Subject	Sf Range	Mole lipids per 100 Amino Acid Residues				
			FC	CE	PL	TG	PR
Type I postprandial	H H	Sf 20 — 400	16	5	31	175	100
Type IV postprandial	O W	Sf 20 — 100	14	29	23	61	100
Normal postprandial	A G	Sf 20 — 100	10	9	17	73	100
Normal alimentary	A G	Sf 20 — 100	15	15	18	63	100

See Table A for abbreviations

rations, also total LP protein was quantitated. The distribution was expressed either as the absolute amount of total lipid in mg per 100 cc of serum or as the per cent of the sum of total lipid in all VLD-LP subfractions.

The per cent total lipid distribution was very similar whether the pattern was estimated on unwashed or washed preparations (Table D).

In fat-induced hyperlipemia, type I, (patient H H) the total lipid pattern showed a marked difference from that of the normal subject, with an absolute (Figure C) and relative maximum (Table E) in fractions A and B, the "chylomicron fractions". Identical distribution was seen in two siblings, S H and L F, with fat-induced hyperlipemia (data by Alaupovic and Furman, personal communication). This type of hyperlipemia satisfies the term hyperchylomicronemia suggested by Furman et al (69). The lipid particles of fraction A isolated from these subjects showed a lipid-protein

composition similar to that of normal alimentary serum chylomicrons (Table J, Chapter IV). The increased fat particles in fat-induced hyperlipemia have been shown previously to have physical properties in common with normal chylomicrons (25, 66).

In subjects (O W, M A, and O C) with carbohydrate-induced hyperlipemia, type IV, a different total lipid pattern in the VLD-LP was observed. The "peak" was within Sf 100—400, i.e. fraction C (Figure C, Table E). It should be stressed, however, that on different occasions in the same subject, variations occurred among the three major fractions i.e. fractions B, C and D, in this type of hyperlipemia (Table F). On the other hand, in type I very good reproducibility was the rule. These variations in relative total lipid distribution seemed to reflect diet and food intake on the days preceding the test. The present data in the normal subject and in subjects with hyperlipemia type IV, agreed with the

Table E Per cent total lipid distribution among VLDL lipoprotein fractions. Comparison between that in three types of hyperlipemia and in the normal subject

Type of Hyperlipemia	Subject	Per Cent Total Lipid Distribution				
		Sf 40 000	Sf 5 000	Sf 400	Sf 100	Sf 50
Type I	H H	67.2	25.4	7.4	—	—
Type IV	O W	3.0	—	65.6	23.4	8.0
	O C	10.1	—	68.4	17.0	4.5
	M A	17.9	—	45.2	35.5	2.4
Type V	R T	19.6	—	45.5	17.3	17.6
	G T	46.5	—	41.3	7.2	5.0
Normal	A G	10.7	11.8	18.7	58.8	—

Table J Lipid and protein composition in human serum and chyle chylomicrons Data expressed as mole lipids per 100 amino acid residues

Source	Type of Hyperlipemia	Sf Range	Mole Lipids per 100 Amino Acid Residues				
			FC	CE	PL	TG	PR
Serum	Type I postprandial	Sf > 5000	55	65	58	1060	100
	Type I postprandial	Sf 400 - 5000	54	67	62	659	100
	Normal alimentary	Sf > 5000	29	29	47	1760	100
	Normal alimentary ¹⁾	~ Sf > 400	10	12	54	450	100
Chyle	"Cream" ²⁾	~ Sf > 400	48	59	440	8250	100
	Crude oil ²⁾	~ Sf > 400	5	16	74	2170	100

See Table A for abbreviations

¹⁾ Composition data from Wood et al 1964 (142)

²⁾ Thoracic duct chyle Composition data from Zilversmit 1965 (80)

hand lacked this protein moiety (76) The finding of β LP protein in a fraction, as in Sf 400—5000 of most hyperlipemic sera, would therefore suggest a contamination of VLD LP This was true for normal alimentary sera In studies by Scanu and Page (56) the presence of β LP protein was eliminated by purification of the chylomicron fraction

When different normal chylomicron fractions were compared the Sf > 5000 and the Sf 400—5000 of the hyperchylomicronemic sera agreed in composition with that of normal, alimentary serum chylomicrons, Sf > 5000 (Table J) When serum chylomicrons of the present study were compared with those isolated in other laboratories (Table J) the agreement in composition was good although differences in cholesterol

content were observed Even qualitatively²⁾ the phospholipids were similar in hyperchylomicronemic and normal, alimentary chylomicrons (Table K) In the same table are included, for comparison, the phospholipid distribution of other serum LP The chylomicrons appear to be closer to α than to β -LP

Chyle chylomicrons and serum chylomicrons differ as to composition A growing body of evidence points to drastic changes in the chyle chylomicrons when they enter the blood stream On *in vitro* incubation of dog chyle chylomicrons with serum the re isolated chylomicrons showed increase in free cholesterol and decrease in triglyceride and phospholipid (although a gain in lyssolecithin was observed) (78) Human chyle chylomicrons also gain in protein content, from 0.2—0.6% (76, 79, 80) to 1% (Paper I) Changes in electrophoretic mobility have also been observed (25, 81) Complete composition

²⁾ The phosphatides were isolated and quantitated by the method of Wre Waterhouse and Marinetti (77)

Table H Lipid and protein composition in VLD lipoproteins, $S_f > 400$ (chylomicron fractions) in various types of hyperlipemia Data expressed as mole lipids per 100 amino acid residues

Type of Hyperlipemia	Lipoprotein Fraction		Mole Lipids per 100 Amino Acid Residues				
	Subjects	S_f Range	FC	CE	PL	TG	PR
Type I : postprandial	HH ¹⁾	S_f 400 - 5 000	54	67	62	659	100
Type IV, postprandial	MA + OC ²⁾	$S_f > 400$	54	55	41	379	100
Type V postprandial	GT + RT ²⁾	$S_f > 400$	63	118	41	417	100

See Table A for abbreviations

¹⁾ Average of four experiments

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CHAPTER IV

The chylomicrons, characterization and composition in various states

Chylomicrons, the main vehicle in the exogenous fat transport, still lack an exact method for their isolation. A variety of flotation procedures have been employed to separate them from the opalescent, triglyceride-rich VLD-LP (20).

Originally, Lindgren et al (73), suggested as chylomicrons in the normal, alimentary serum, a fraction floating at D 1.006 at a centrifugal force 0.1×10^6 g/min. Oncley (50) characterized the normal chylomicrons as LP of S_f 10,000 \pm 5,000.

The fatty acid composition (74) of the lipids of the fraction $S_f > 400$ (estimated by this author) from the normal, alimentary serum, showed good agreement with that of the ingested fats. When LP of lower S_f value were included, the fatty acid discrepancies increased markedly (75).

In the present study, the presence in hyperchylomicronemia of large amounts of "particulate fat" (26) in fractions $S_f > 5000$ and S_f 400-5000, and the identical composition of these two fractions (Table J), indicated that in this lipid disorder (with low amounts of VLD-LP), $S_f > 400$ contained only chylomicrons.

In other types of hyperlipemia the task of separating chylomicrons from VLD-LP was more difficult (20, 72). In type V, the $S_f > 400$ contained LP different in composition to both VLD-LP and chylomicrons (Table H).

Another way of characterization and identification of chylomicrons may be through their protein moiety. The VLD-LP appear to be characterized by the presence of β -LP protein (Chapter V). Pure chyle chylomicrons, on the other

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¹⁾ Composition data from Wood et al. 1964 (142)

²⁾ Thoracic duct chyle Composition data from Zilversmit, 1965 (80)

hand lacked this protein moiety (76). The finding of β -LP protein in a fraction, as in Sf 400—5000 of most hyperlipemic sera, would therefore suggest a contamination of VLD LP. This was true for normal alimentary sera. In studies by Scanu and Paape (36) the presence of β LP protein was eliminated by purification of the chylomicron fraction.

When different normal chylomicron fractions were compared, the Sf > 5000 and the Sf 400—5000 of the hyperchylomicronemic sera agreed in composition with that of normal, alimentary serum chylomicrons Sf > 5000 (Table J). When serum chylomicrons of the present study were compared with those isolated in other laboratories (Table J) the agreement in composition was good although differences in cholesterol

content were observed. Even qualitatively^{*)} the phospholipids were similar in hyperchylomicronemic and normal, alimentary chylomicrons (Table K). In the same table are included, for comparison, the phospholipid distribution of other serum LP. The chylomicrons appear to be closer to α than to β LP.

Chyle chylomicrons and serum chylomicrons differ as to composition. A growing body of evidence points to drastic changes in the chyle chylomicrons when they enter the blood stream. On *in vitro* incubation of dog chyle chylomicrons with serum, the re-isolated chylomicrons showed increase in free cholesterol and decrease in triglyceride and phospholipid (although a gain in lysolecithin was observed) (78). Human chyle chylomicrons also gain in protein content, from 0.2—0.6% (76, 79, 80) to 1% (Paper I). Changes in electrophoretic mobility have also been observed (25, 81). Complete composition

^{*)} The phosphatides were isolated and quantitated by the method of Nye, Waterhouse and Marinetti (77).

data of human thoracic duct chylomicrons has recently been reported in cream and corn oil feeding experiments (80). The differences in these chyle chylomicrons and the serum chylomicrons (Table J) correspond to the differences that could be expected from the *in vitro* studies.

Table K *Phospholipid composition of serum lipoproteins*

Protein Fraction		No of Samples	Per Cent of Total Lipid Phosphorus					Reference
			Lecithin	Sphingo- myelin	Lyso- lecithin	Phosphatidyl Ethanolamine	Unknown	
Chylomicrons ¹⁾	Sf > 5 000	6	79	9.1	—	3.6	7.7	Present study
Chylomicrons ²⁾	Sf > 400	12	78.5	11.7	4.2	5.6	—	Wood et al. 1960
LDL	Sf 20 — 400	5	74.0	18.3	—	7.7	—	Nelson and Freese 1960 (143)
	Sf 11 — 20	5	68.8	25.6	—	5.6	—	Nelson and Freese 1960 (143)
	D 1.063 — 1.21	5	80.8	11.8	—	7.4	—	Nelson and Freese 1960 (143)
+ VLDL	D > 1.063	6	69.9	13.6	11.2	5.2	—	Phillips 1959 (144)

¹⁾ obtained from three siblings with type I hyperlipemia

²⁾ obtained from six normal subjects in the absorptive state

Protein moieties of the VLD-lipoproteins

CHAPTER V

Isolation and characterization of phospholipid-protein residues (Papers IV and V)

The method of lyophilization in the presence of starch, and lipid extraction with heptane, was utilized for the partial delipidization of serum LP (Chapter II). By this method neutral lipid free residues with characteristic phospholipid-protein ratios were obtained from any serum LP. When characterized by analytical ultracentrifugation the water-soluble residue appeared as a schlieren pattern containing one or several of three sedimenting boundaries with approximate sedimentation coefficients 4S, 7S and 14S.

Partially delipidized α LP, showed the 4S residue as a single homogeneous boundary (Figure 2A). The corrected sedimentation coefficient $S_{20,w}^0$ was 4.3S and the hydrated density 1.17 g/cc (Table I). After partial lipid extraction the β LP Sf 0—20, gave rise to a 14S residue. This had a characteristic appearance (Figure 2B) and a tendency to aggregation causing minor faster moving products (20S, 27S) to appear. The $S_{20,w}^0$ for the 14S residue was 14.5S (Table I) not taking into account the Johnston Ogston effect of the aggregate products.

Partial delipidization of narrow and broad LP fractions of the VLD LP, Sf 20—400, gave evidence for three over-

lapping residues with sedimentation characteristics 4S, 7S and 14S. This finding is in agreement with the suggestion of heterogeneity in VLD-LP (Paper I). Interestingly, after ether extraction of the VLD-LP a 4S and a 14S residue was identified in the analytical ultracentrifuge, but only unconstantly a 7S residue. This is possibly due to the vulnerability of the 7S residue to the vigorous ether extraction procedure (Paper II).

The mixture of three residues in partially delipidized VLD LP prompted efforts toward their separation and isolation in sufficient amounts for complete lipid and protein characterization.

Electrophoresis on various media, paper, cellulose acetate and starch gel (Figure I) showed that the three residues had a characteristic mobility, and that each was different from the others. On starch block electrophoresis the 4S, the 7S and the 14S residues moved approximately, with α -1, α -2 and β -globulins, respectively. Due to difficulties in the recovery of the phospholipid-protein material from starch powder (also experienced by Kunkel and Trautman) (82) a synthetic product, *Perikon* C-870 (Stockholm Superfosfat AB, Stockholm,

Sweden), was used for preparative purposes. In this medium, a powdered co-polymer of polyvinyl chloride and polyvinyl acetate, the residues showed the same electrophoretic mobility and even sharper separation due to less endosmosis (83). The recovery of water-soluble phospholipid-protein material from the *Pevikon* was 50–60 per cent protein by weight.

The electrophoresis on *Pevikon* block frequently allowed the isolation in relatively pure form of the 14S from the 4S and the 7S residues (Figure 1). The 4S and the 7S residues, however, were not completely separated and were therefore eluted together and subsequently isolated by preparative ultracentrifugation.

The residues were characterized in the analytical ultracentrifuge by their values of $S_{20,w}^0$, hydrated density (Table I) and in immunoelectrophoresis and diffusion for their antigenic properties by specific anti-sera and by commercially available antibodies to human serum. The protein moiety, the apolipoprotein^{*}, of each residue was (after denaturation) identified by its N-terminal amino acids and by its peptide patterns ("fingerprint") (Figures 4 and 5). The phospholipid portion of each residue was characterized by its phosphatide distribution (Table III). It could be concluded from these data that the 4S residue (and its protein moiety) isolated from the VLD-LP was

identical to the 4S residue of the α -LP, containing apolipoprotein A. Furthermore, the 14S residue, and its protein moiety, obtained from VLD-LP was identical in physical and chemical characteristics with the 14S residue of the β -LP, containing apolipoprotein B (Tables I and II).

The 7S residue was characteristic of the VLD-LP. The $S_{20,w}^0$ found for the 7S residue was 6.9S and the hydrated density was 1.09 g/cc (Table I). Figure D shows the extrapolation of the inverted value of the sedimentation coefficients ($1/S_{20,w}^0$) to infinite dilution ($1/S_{20,w}^{\infty}$). The extrapolation of the hydrated density from the sedimentation coefficients at three solvent densities (as described in Paper V) is shown in Figure G (Chapter IX). Similar physical data were obtained for this residue when isolated from LP of Sf > 400 where it occurred singly, exclusively in sera of hyperlipemia type V. The phospholipid-protein ratio (weight by weight) of the 7S residue, 2.4, was higher than that for

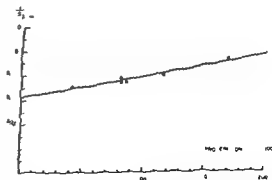


Figure D. The inverted value of the sedimentation coefficient ($1/S_{20,w}^0$) of the 7S residue at different protein concentrations (in mg per 100 cc). Extrapolation to zero concentration gives the inverted value of the sedimentation coefficient at infinite dilution ($1/S_{20,w}^{\infty}$).

* The term "apolipoprotein," as suggested by Oncley (144), designates the lipid free protein moiety of a lipoprotein. Thus apolipoprotein A designates the protein moiety of α lipoprotein, apolipoprotein B the protein moiety of β lipoprotein, and apolipoprotein C the protein moiety of a lipoprotein present in VLD lipoproteins (Sf > 20).

the 4S and the 14S residues, 0.8 and 0.7 respectively. The high phospholipid content gave the 7S residue a large schlieren peak in the analytical ultracentrifuge (Figure 2C). Further heptane extraction of the LP fraction ($S_f > 400$) containing the 7S residue, did not reduce the phospholipid protein ratio, nor did it cause any apparent change in the physical characteristics of the residue. The protein moiety of the 7S residue, the apolipoprotein C, gave one precipitation reaction with specific antisera, showed two N-terminal amino acids, serine and threonine (Table II) and had peptide patterns different from those of the other two apolipoproteins (Figures 4 and 5).

The distribution of the residues among the VLD LP studied by analytical ultracentrifugation of partially delipidized subfractions, revealed that the 4S residue was present in chylomicrons (in fraction $S_f > 5000$ of hyperchylomicronemia as

a single component) and in the least dense VLD LP. The 14S residue was characteristic for the heavier VLD LP at the right end of the VLD LP spectrum. The 7S residue, finally, was found within the intermediate chylomicron and VLD LP fractions. The distribution of the apolipoproteins according to Alaupovic, Ledford and Furman (to be published) is given in Figure E.

The relative proportion of the three residues and indirectly the apolipoproteins was evaluated by protein determinations of the residues recovered from the block electrophoresis. This method assumed an equal recovery of all three residues. In type IV hyperlipemia the partially delipidized VLD-LP ($S_f > 20$), contained mainly apolipoprotein B, 70 per cent, with about equal amounts of apolipoproteins A and C, 20 per cent and 10 per cent, respectively. The type V hyperlipemia, was charac-

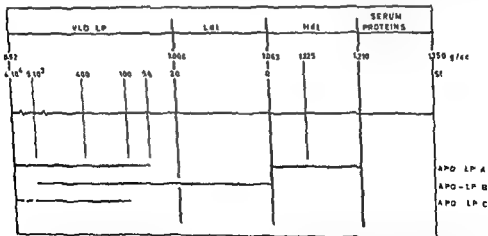


Figure E. The serum LP spectrum. Distribution of the three LP protein moieties, apolipoproteins A, B and C among the serum LP fractions. For the VLD LP, determinations were done on hyperlipemic sera. Data obtained from P. Alaupovic, J. Ledford and R. H. Furman, to be published.

terized by the presence of more 7S residue, apolipoprotein C. The relative proportions of the three apolipoproteins were 15 per cent, 50 per cent and 35 per cent for apolipoproteins A, B and C, respectively (Table IV). Normal sera showed evidence for the presence of all three apolipoproteins, but their relative proportions could not be studied because the amounts recovered were too little. It could be expected, however, from the total lipid distribution with mainly VLD-LP, Sf 20–100, (Chapter III) that apolipoprotein B would be predominant in a fasting normal subject.

No exact values for molecular weights of the lipid-free apolipoproteins could be obtained from the data. However, some information on their size may be deduced from the relative proportion of the protein moiety in the molecular weight of the residue. The experimentally determined molecular weights of the 4S and the 7S residues were 172,000 and 834,000 respectively (Table L). The Stoke minimum molecular weight (Paper

I, p 598) assuming a spherical particle, calculated from $S^{0}_{20,w}$ and hydrated density, gave for the 14S residue a value of 1,040,000. These molecular weights of the residues were of the order of magnitude expected from thin-layer chromatography on Sephadex G-200 according to the method by Johansson and Rymo (84).

From these data the molecular size of the protein portion of the residues was estimated to be 100,000, 600,000 and 250,000 for apolipoproteins A, B, and C, respectively (Table L). In comparison, the direct determination of the molecular weight of the nearly lipid-free protein moiety of α -LP, the apolipoprotein A, has given values of 75,000 (57) and 36,000 (85), the latter being a monomer of the former.

The β -LP protein, apolipoprotein B, has been found to contain two identical peptide chains of approximate molecular weight 380,000 each (86). The estimated value in the present study agreed fairly well with this data.

Table L. Molecular weights of lipoprotein residues and apolipoproteins

Lipoprotein Fraction		Residue						Apolipoprotein		
	Molecular Weight	Protein Content (%)	S_{20}^0 (S)	Hydrated Density (g/cc)	Molecular Weight		Approximate Molecular Weight			
					Measured	Estimated ¹⁾	Measured	Estimated		
LP Hdl 2	375 000 ²⁾	4 S	55	4.3	1.17	172 000	120 000	A	100 000	70 000
Hdl 3	175 000 ²⁾									
LP	2 300 000 ²⁾	14-S	59	14.0	1.13	—	1 040 000	B	—	600 000
		7 S	30	6.9	1.09	834 000	600 000	C	250 000	180 000

Assuming a spherical particle. For calculations see Paper I p 598.

Lipoprotein molecular weight from Hazelwood 1958 (87).

Lipoprotein molecular weight from Oncley 1963 (50).

A value of 250,000 for the protein portion of the apolipoprotein C₂ would indicate, if the two peptide chains representing N terminal serine and threonine are of equal size a molecular weight of 125,000 (or fractions thereof) for each

peptide. The difference in estimated and experimentally determined molecular weights, 600,000 and 834,000 respectively, may indicate that the 7S residue has an ellipsoid rather than spherical shape.

Discussion

CHAPTER VI

The structure of the VLD and the β -lipoproteins

Only limited information is available regarding the structure of serum LP. Lipid extraction experiments in the present study (Chapter II) supplied certain suggestions as to the lipid-protein and lipid-lipid interactions in the LP. On the basis of these findings a hypothetical LP model has been proposed. Estimations were carried out assuming an ideal monomolecular distribution in the surface layer of the micellar particle.

Of the four LP classes, the α -LP diverge most from the other serum LP, particularly in shape (87), and in lipid binding properties (Chapter II) (88). A growing body of evidence points to α -LP as a macromolecular compound (64, 89).

The β -LP, on the other hand, represent a micellar form of serum LP. Estimations of the LP structure were based on this LP, assuming a spherical particle (90). Studies on the protein moieties of the serum LP, indicated the presence of triglyceride-rich β -LP within the heterogeneous VLD-LP. Calculations were therefore also applied to the four VLD-LP subfractions. Even data on chylomicrons, $S_f > 5000$, were included. Chylomicrons have been established as true LP (Paper I) but contain α rather than β -LP protein (Chapter V).

In view of solubility properties, the LP show the characteristics of a protein. This has been taken to indicate that the protein moiety is present at or near the LP surface and possibly makes up an outside shell of the LP structure (62, 89, 91). From the present data (Table M) it appears, however, that there is not enough protein in any VLD or β -LP to make a monomolecular layer, minimum thickness 7\AA (92) around the entire LP molecule. The surface area occupied by protein was estimated from the number of amino acid residues per LP molecule (Table A) and the value for the area per residue. At an oil-water interface a film of 1 mg protein would occupy 0.7 m^2 , or 13.6 \AA^2 per amino acid residue (93). A value of 0.6 m^2 has also been reported (94).

With the protein interspersed with phospholipid, a larger area of the LP surface would be occupied. The polar phospholipid, could also be expected to orient to the surface of the LP. Lipid extraction experiments (Chapter II) postulated a strong bond between phospholipid and the protein moiety. Furthermore, the phospholipid appeared to serve a function in stabilizing the protein

Table M Lipoprotein total surface area compared with the area covered by available protein protein phospholipid and protein phospholipid cholesterol in monomolecular films Computed data are given for the average lipoprotein of five VLD lipoprotein fractions and the β -lipoproteins

Lipoprotein Fraction	Lipid protein Area per g Mol Wt of LP				
	Diameter (Å)	Surface Area ^{a)} (Å ²)	PR ^{b)} (Å ²)	PR + PL ^{c)} (Å ²)	PR + FC + PL ^{d)} (Å ²)
Fraction A	> 2572	> 2.1 × 10 ¹⁰	0.6 × 10 ⁹	1.7 × 10 ⁹	2.6 × 10 ⁹
Fraction B	922	2.6 × 10 ⁹	0.5 × 10 ⁹	1.3 × 10 ⁹	1.9 × 10 ⁹
Fraction C	445	5.8 × 10 ⁸	1.7 × 10 ⁸	3.4 × 10 ⁸	4.2 × 10 ⁸
Fraction D	366	3.9 × 10 ⁸	1.6 × 10 ⁸	2.7 × 10 ⁸	3.2 × 10 ⁸
Fraction E	275	2.4 × 10 ⁸	0.9 × 10 ⁸	1.3 × 10 ⁸	1.5 × 10 ⁸
β -LP ^{e)}	192	1.0 × 10 ⁸	0.6 × 10 ⁸	0.9 × 10 ⁸	1.0 × 10 ⁸

See Table A for abbreviations

^{a)} LP surface area = $\pi (d-14)^2$ where d is LP diameter in Å

^{b)} The value used for av area per amino acid residue 13.6 Å² (0.7 m² per mg protein) was obtained at an oil water interface and 20 dynes per cm film pressure (93)

^{c)} The value used for mean area per molecule of PL was 41.5 Å² (96)

^{d)} The value used for mean area per molecule of FC and PL in a mixed film was 37 Å² (96)

^{e)} Calculated from data of Oncley 1963 (50)

moiety (Chapter II) as well as the whole LP (95)

Calculations for the phospholipid area were done as for the protein with composition data taken from table A For the average area per molecule in a film of lecithin a value of 41.5 Å² (96) was used The computations show that there is not enough of phospholipid and protein to cover the LP molecule with a monomolecular layer in any of the VLD or β LP (Table M)

Free cholesterol is known to form with phospholipid mixed films that are more compact than the pure phospholipid films Phospholipid cholesterol associations occur commonly in living tissues particularly in cell membranes (97) Consecutive lipid extractions of β LP (Chapter II) revealed that free cholesterol was definitely harder to re-

move than the other neutral lipids, triglyceride and cholesterol ester This finding was evidence in favour of a strong association between free cholesterol and the phospholipid protein complex or its constituents The suggestion of free cholesterol and phospholipid appearing with the protein moiety at the LP surface seems to be supported by lipid exchange studies Using labelled compounds phospholipid (98) and free cholesterol (99) exchanged freely among LP and corpuscular elements in the blood (100) This was not true for triglyceride and cholesterol ester

On the basis of these indirect evidence it was assumed that free cholesterol and phospholipid form a mosaic in the film of protein The surface area occupied by this association was estimated as above from composition data in table A A value

of 37 Å² (96) was used for the area per molecule cholesterol and phospholipid in the mixed film (approximate molecular proportion 1:1). This combination of protein, phospholipid and free cholesterol (Table M) theoretically suffice to form a monomolecular distribution around the β-LP and possibly the chylomicrons. In the latter, however, it should be stressed that the minimum LP surface area is given. From these estimations the VLD-LP were suggested to contain a "loose" surface layer lacking the ideal monomolecular distribution due to large size, insufficient amounts of membrane constituents, or both. The significance of this "loose" membrane in the stability and metabolism of VLD-LP is an intriguing question that will be further touched upon in connection with the LP-metabolism.

Data in Table A, and the approximate molecular volume of tristearate, 1600 Å³, and cholesteryl stearate, 1100 Å³, allowed estimations of the total volume of the packed molecules of triglyceride and cholesterol ester, in the LP. The values used for the molecular volumes were in agreement with those found in the crystalline state 1493 Å³ and 1120 Å³ for tristearate and cholesteryl stearate, respectively (S. Abrahamsson and K. Larsson, personal communication). It could be established that the space inside the surface membrane, with a diameter of d-14 Å, was large enough for the packed molecules of cholesterol ester and triglyceride (Table N). Even enough additional space, about 10% of the total volume, was left to allow a liquid distribution of these molecules. It is apparent, however, that this "inner" space could

Table N. Lipoprotein total volume compared with the volume of packed molecules of available cholesterol ester and triglyceride. Computed data are given for the average lipoprotein of five VLD lipoprotein fractions and the β lipoprotein.

Lipoprotein Fraction	Volume of Packed Lipid Molecules per g Mol Wt of LP				
	Diameter (Å)	Volume ¹⁾ (Å ³)	CE ²⁾ (Å ³)	TG ³⁾ (Å ³)	CE + TG (Å ³)
Fraction A	> 2572	> 8.7 × 10 ⁹	0.3 × 10 ⁹	8.0 × 10 ⁹	8.3 × 10 ⁹
Fraction B	922	3.9 × 10 ⁸	0.3 × 10 ⁸	3.4 × 10 ⁸	3.7 × 10 ⁸
Fraction C	445	4.2 × 10 ⁷	0.5 × 10 ⁷	3.3 × 10 ⁷	3.8 × 10 ⁷
Fraction D	366	2.3 × 10 ⁷	0.3 × 10 ⁷	1.6 × 10 ⁷	1.9 × 10 ⁷
Fraction E	275	9.3 × 10 ⁶	1.4 × 10 ⁶	6.1 × 10 ⁶	7.5 × 10 ⁶
β LP ⁴⁾	192	2.9 × 10 ⁶	1.4 × 10 ⁶	0.5 × 10 ⁶	1.9 × 10 ⁶

See Table A for abbreviations.

¹⁾ Volume = $\pi \frac{(d-14)^3}{6}$ where d is lipoprotein diameter in Å.

²⁾ The value used for molecular volume of cholesteryl stearate 1100 Å³ was obtained from mol wt (654) density (0.99 g/cc) and the Avogadro number.

³⁾ The value used for molecular volume of tristearate 1600 Å³ was obtained from vol wt (893) density (0.92 g/cc) and the Avogadro number.

⁴⁾ Calculated from data of Oncley 1963 (50).

not accommodate any large quantity of LP water, as suggested in earlier LP models (89). Recently the water content of β LP has been established as less than 10 % by weight (50). The water content of the VLD LP appears to be of the same order of magnitude, as judged from agreement in hydrated and anhydrous density (Paper I). This relatively low water content would mainly account for the hydration of the protein moiety. In this connection the water molecules would be lodged with the protein moiety, possibly extending perpendicularly out from the LP surface (101).

Interestingly, Zilversmit (102), in studies on dog chyle chylomicrons found evidence for a similar LP structure. The disrupted and isolated membranes ap-

peared to contain protein, phospholipid, free cholesterol and a few per cent triglyceride. The chylomicron membrane surrounded an 'oil phase', composed of cholesterol ester and triglyceride.

Vandenheuvel (89) in 1962 suggested a β -LP model, that shows marked differences from the one now suggested. His model was based on a 60 % (by weight) LP water content with the major portion lodged as a central water core. A spherical protein shell of 7 Å thickness was estimated to surround and cover the LP molecule, assuming an area of 35–38 Å² per amino residue. All lipids were accommodated between the protein layer and the water core, in a 22 Å wide annular space.

CHAPTER VII

Chylomicron metabolism

By definition, chylomicrons originate in the intestines. Ninety per cent of the neutral fat content of chyle chylomicrons comes from ingested fat (103) while the major portion of the phospholipid content is of endogenous source (103). This latter finding indicates that phospholipids are either synthesized in the intestinal wall or transported there from the serum. As to the protein moiety the same two possibilities of origin, intestinal derivation (79, 104, 105) and serum contribution (69) have been discussed.

Assuming that the α LP protein (76) even in man is the major and characteristic protein moiety of the chyle

chylomicrons, it can be estimated that a biosynthesis of this protein in the intestinal mucosa could fill the need also for α LP. A rate of 100 g chylomicron fat influx per day and a 0.5 per cent protein content in chyle chylomicrons (Chapter IV) would give a gain of 0.5 g of α LP protein in the serum daily. With a total amount of 3–4 g α LP protein in the serum and a half life of about 4 days (69) this would be enough to be the single source of α LP protein. On the other hand, much evidence indicates a serum contribution of the serum chylomicron protein. When ¹³¹I-labelled α -LP protein was injected into man, the

of 37 Å² (96) was used for the area per molecule cholesterol and phospholipid in the mixed film (approximate molecular proportion 1:1). This combination of protein, phospholipid and free cholesterol (Table M) theoretically suffice to form a monomolecular distribution around the β LP and possibly the chylomicrons. In the latter, however, it should be stressed that the minimum LP surface area is given. From these estimations the VLD LP were suggested to contain a "loose" surface layer lacking the ideal monomolecular distribution due to large size, insufficient amounts of membrane constituents, or both. The significance of this "loose" membrane in the stability and metabolism of VLD LP is an intriguing question that will be further touched upon in connection with the LP metabolism.

Data in Table A, and the approximate molecular volume of tristearate, 1600 Å³, and cholesteryl stearate, 1100 Å³, allowed estimations of the total volume of the packed molecules of triglyceride and cholesterol ester, in the LP. The values used for the molecular volumes were in agreement with those found in the crystalline state 1493 Å³ and 1120 Å³ for tristearate and cholesteryl stearate, respectively (S. Abrahamsson and K. Larsson, personal communication). It could be established that the space inside the surface membrane, with a diameter of d=14 Å, was large enough for the packed molecules of cholesterol ester and triglyceride (Table N). Even enough additional space, about 10% of the total volume, was left to allow a liquid distribution of these molecules. It is apparent, however, that this 'inner' space could

Table N Lipoprotein total volume compared with the volume of packed molecules of available cholesterol ester and triglyceride. Computed data are given for the average lipoprotein of five VLD lipoprotein fractions and the β lipoprotein.

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Fraction B	922	3.9 × 10 ⁸	0.3 × 10 ⁸	3.4 × 10 ⁸	3.7 × 10 ⁸
Fraction C	445	4.2 × 10 ⁷	0.5 × 10 ⁷	3.3 × 10 ⁷	3.8 × 10 ⁷
Fraction D	366	2.3 × 10 ⁷	0.3 × 10 ⁷	1.6 × 10 ⁷	1.9 × 10 ⁷
Fraction E	275	9.3 × 10 ⁶	1.4 × 10 ⁶	6.1 × 10 ⁶	7.5 × 10 ⁶
β LP ⁴⁾	192	2.9 × 10 ⁶	1.4 × 10 ⁶	0.5 × 10 ⁶	1.9 × 10 ⁶

See Table A for abbreviations.

¹⁾ Volume = $\pi \frac{(d-14)^3}{6}$, where d is lipoprotein diameter in Å.

²⁾ The value used for molecular volume of cholesteryl stearate, 1100 Å³ was obtained from mol. wt. (654) density (0.99 g/cc) and the Avogadro number.

³⁾ The value used for molecular volume of tristearate 1600 Å³ was obtained from mol. wt. (893) density (0.92 g/cc) and the Avogadro number.

⁴⁾ Calculated from data of Oncley 1963 (50).

Table O Hypothetical transformation of chylomicrons into α lipoproteins Comparison of computed conversion steps compared with that of experimental fractions Data expressed as mole lipids per 100 amino acid residues

Lipoprotein Fraction		LP Density (g/cc)	Mole Lipids per 100 Amino Acid Residues				
Hypothetical Transformation	Experimental Fraction		FC	CE	PL	TG	PR
Chylomicrons	Fraction A	0.928	55	65	58	10.0	100
TG Hydrolysis I		0.936	55	65	■	650	100
	Fraction B	0.936	49	73	55	590	100
Protein Dimerization I		0.941	28 (55)	33 (65)	29 (58)	325 (650)	100 (200)
TG Hydrolysis II		0.957	28 (55)	33 (65)	29 (58)	169 (337)	100 (200)
	Fraction C	0.957	22	37	33	170	100
Protein Dimerization II		0.979	14 (55)	16 (65)	15 (58)	84 (337)	100 (400)
	Fraction D	0.977	13	26	23	84	100
TG Hydrolysis III		1.083	14 (55)	16 (65)	15 (58)	3.3 (13)	100 (400)
	Idl 2)	1.09	6	12	13	3.2	100

See Table A for abbreviations

¹⁾ Composition data from Oncley and Gurd 1953 (145)

LP density (D_{LP}) as related to changes in mole lipid composition*)

This relationship in the case of chylomicron degradation was

$D_{LP} =$

$$22.6 + 42.1 + 43.6 + 0.817X + 0.156Y$$

$$21.3 + 42.5 + 45.0 + 0.893X + 0.117Y$$

where X is the triglyceride and Y the amino acid residue content. For Y a value of 100, 200 and 400 was used for the original fraction and the two polymerization steps, respectively. Protein dimerization steps were chosen at appropriate levels and the degree of triglyceride hydrolysis chosen to match

the density of the experimental fractions. The interesting agreement in composition of the estimated products and the experimental fractions is demonstrated in Table O. Numbers in parentheses correspond to the data in Figure F in which the metabolism of chylomicrons into α LP is visualized. (The dashed curve, represents a conversion by triglyceride removal alone). In the last hydrolysis step where the LP increase in density from 0.97 to 1.09 g/cc, only a relatively small amount of triglyceride (from 84 to 3 mole per 100 amino acid residues) have to be hydrolyzed as compared to the earlier steps. Thus a rapid last metabolic step may explain the lack of chylomicron protein, α -LP protein within this density range. However, other possi-

*) Molecular weights and values of density of lipid components and amino acid residues are given in Table A and Paper I page 598 respectively.

chylomicron appearing in serum after fat-feeding contained a high portion of labelled α -LP protein (69). This would suggest that the newly synthesized chylomicrons contain protein also from the serum pool.

The chylomicrons enter the blood stream by way of the thoracic duct. In serum the chylomicrons change in chemical composition and physical characteristics, among other changes there is a gain in protein content and loss in triglyceride moiety (Chapter IV). Their metabolism is rapid. In man, the serum chylomicrons have a half-life of 17 minutes (106). It is suggested from *in vivo* studies (28, 107, 108), that the serum chylomicron goes through a stepwise reduction in size and increase in density, through the loss of lipids, predominantly glycerides. The glyceride moiety is hydrolyzed through the action of LP lipase into glycerol and fatty acids. The fatty acids released are transported as a complex with albumin (109, 110). The appearance of mono- and diglycerides (111, 112) as well as fatty acids (113) in serum during chylomicron clearing support these concepts. In regard to their protein moiety (Chapter V), it may well be assumed that chylomicrons metabolize into α -LP. During chylomicron clearing in man (107, 114), as well as in the dog (115), an increase in α -LP lipid has been established. However the absence of α -LP or α -LP protein within the β -LP region, D 1006—163, has been used as an argument against a successive metabolism of chylomicrons into α -LP.

In the present study, the molar com-

position data (Table B) in fractions containing α -LP protein, i.e. fractions A through D, show that if an interconversion among these LP occurs this cannot be caused by triglyceride removal alone but by loss of cholesterol and phospholipid, as well. The fate of cholesterol and phospholipid moiety released under such circumstances is hard to explain with our present knowledge of LP. Thus other interpretations have to be searched for. The difference in protein and triglyceride content at the conversion of the chyle chylomicrons into serum chylomicrons (Chapter IV), suggested the possibility of a protein uptake or polymerization in addition to triglyceride removal. α -LP and its protein moiety possess good qualifications for this function, because they have a pronounced tendency to polymerization (85, 116) a high affinity for fat emulsions (56) and the specific ability to activate substrates for LP lipase (56, 117).

Composition and density of chylomicron degradation products obtained by protein uptake and successive triglyceride removal, was estimated and compared (Table O) with the actual data of experimental fractions B through D (in which α -LP protein was present).

The equation,

$$D_{FC} \times m_1 \times MW_{FC} + D_{CE} \times m_2 \times MW_{CF} + D_{FL} \times m_3 \times MW_{FL} + D_{TG} \times m_4 \times MW_{TG} + D_{PL} \times m_5 \times MW_{PL} = D_{IF} \times (m_1 \times MW_{IF} + m_2 \times MW_{CL} + m_3 \times MW_{FL} + m_4 \times MW_{TG} + m_5 \times MW_{PL})$$

where D stands for density, m for molar concentration and MW for molecular weight, was used for the calculation of

CHAPTER VIII

VLD lipoprotein metabolism

VLD LP, Sf 50—100 (121), and Sf > 12 (122) are biosynthesized by the liver. Support for this is given by studies in animals with liver slice techniques (121, 123) and liver perfusion (121, 122, 124). Beta LP protein apolipoprotein II was identified by immunochemistry (121) in the newly synthesized VLD LP. (Compare discussion on identification of protein moieties in Chapter IX). The rate of this LP synthesis is possibly related to factors that control triglyceride biosynthesis, such as fatty acid mobilization from adipose tissue (125, 126) and *de novo* synthesis of fatty acids from carbohydrate (66, 127, 128). The excess of fatty acids is incorporated into triglycerides in the liver (129) and by way of liver triglycerides (130) secreted into the blood stream. The increased serum triglycerides are transported with VLD LP (131) opal

escent β particles (128). In the present study, VLD LP with β LP protein possibly of hepatic origin, were found within Sf 20—5000 in hyperlipemic states (Chapter V).

A conversion of VLD LP from higher into lower flotation rates and ultimately into β LP, through the removal of glycerides, has been suggested from experiments *in vivo* with I^{131} labeled VLD LP (132) and *in vitro* by the lipolytic action of LP lipase in post heparin plasma (133, 134). A metabolism of VLD LP into β LP seems likely from point of view of LP protein distribution (Figure E). Furthermore the mole composition data particularly of fractions D and E as compared to that of β LP suggest a metabolic relationship between these fractions (Table B). Composition and density of intermediate metabolic products were estimated as for the

Table B Hypothetical transformation of VLD lipoproteins into β -lipoproteins. Comparison of computed conversion steps compared with that of experimental fractions. Data expressed as mole % per 100 amino acid residues.

Hypothetical Transformation	Lipoprotein Fraction	Experimental Fraction	Li Density (g/cc)	Mole Lipids per 100 Amino Acid Residues				
				FC	CE	PL	TG	PR
TG Addition II			0.977	11	31	16	83	100
		Fraction D	0.977	11	26	23	84	100
TG Addition I			0.989	11	31	16	61	100
		Fraction E	0.989	11	20	16	61	100
β -LP)			1.03	11	31	16	7	100

See Table A for abbreviations

) Composition data from Oncley (50)

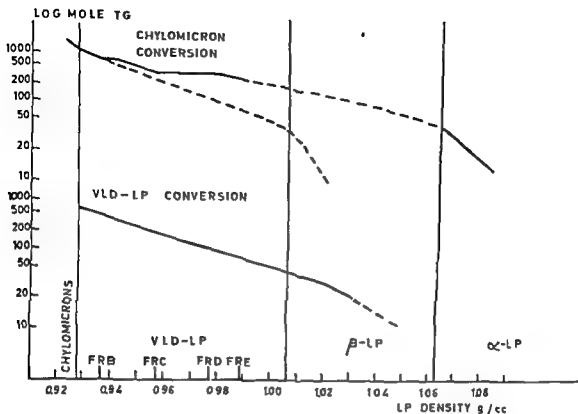


Figure F Hypothetical transformation of chylomicrons and VLD LP into LP of higher density. Change in LP density as related to loss of triglyceride expressed as log mole triglyceride (TG) per 100 amino acid residues. Chylomicron conversion by triglyceride loss alone (dashed curve) and by triglyceride loss and two steps of dimerization (doubling of the protein content) (solid curve). VLD LP conversion by triglyceride loss alone (solid curve). Solid line in the chylomicron conversion (upper curve) indicates fractions containing apolipoprotein A, and in the VLD LP conversion the presence of apolipoprotein B. For numerical data see also Tables O and P.

ble explanations for the " β gap", the lack of α -LP protein within the β -LP region, have to be kept in mind. An extravascular metabolism (118, 119) of the chylomicron degradation products with the concomitant release of α -LP or α -LP protein back into the serum must be considered.

The protein moiety, added to the chylomicron at the successive conversions, would most likely represent α -LP protein in a process of polymerization. In serum, α -LP protein occurs as

a lysolecithin complex (120) in the very-high-density lipoproteins (VHdl) (33, 34). This fraction could be a source of the α -LP protein. However, the presence of phospholipid with the added protein in the chylomicron conversion is not suggested from composition data (Table O). The apolipoprotein C may possibly, under certain circumstances, be an alternate protein moiety, serving in chylomicron metabolism in the form of a phospholipid-protein complex (Chapter IX).

CHAPTER VIII

VLD-lipoprotein metabolism

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escent β particles (128). In the present study, VLD LP with β LP protein possibly of hepatic origin, were found within Sf 20—5000 in hyperlipemic states (Chapter V).

A conversion of VLD-LP from higher into lower flotation rates and ultimately into β -LP, through the removal of glycerides, has been suggested from experiments *in vivo* with 131 labeled VLD LP (132) and *in vitro* by the lipolytic action of LP lipase in post heparin plasma (133, 134). A metabolism of VLD LP into β LP seems likely from point of view of LP protein distribution (Figure E). Furthermore the mole composition data particularly of fractions D and E as compared to that of β -LP suggest a metabolic relationship between these fractions (Table B). Composition and density of intermediate metabolic products were estimated as for the

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Hypothetical Transformation	Lipoprotein Fraction	LP Density (g/cc)	Mole Lipids per 100 Amino Acid Residues				
			FC	CE	PL	TG	PR
TG Addition II		0.977	II	31	16	83	100
	Fraction D	0.977	13	26	23	84	100
FC Addition I		0.989	II	31	16	61	100
	Fraction E	0.989	II	20	16	61	100
β -LP ¹⁾		1.03	II	31	16	7	100

See Table A for abbreviations.

¹⁾ Composition data from Oncley (50).

chylomicron α -LP conversion (Chapter VII), although only triglyceride removal was taken into consideration

Calculations were based on data of the end product, the β -LP, to which triglyceride was added. The equation relating change in LP density (D_{11}) with change in triglyceride was,

$$D_{LP} = \frac{4.7 + 20.4 + 11.9 + 0.817X + 15.5}{4.4 + 20.5 + 12.2 + 0.893X + 11.7}$$

where X is the triglyceride content. This equation is satisfied by the curve in Figure F. The composition of the estimated conversion products agree well with those of the experimental fractions, Table P. Regarding differences in cholesterol ester, see also Chapter I.

No calculations on Sf 100—5000 were made although the presence of β -LP protein (Figure E) would indicate the presence of VLD-LP in these fractions. The occurrence of these LP in hyperlipemic states only (Chapter III) may indicate that they are an abnormal form of LP, not properly metabolized. An imbalance in lipids and protein, through

an excess of triglyceride (from fatty acid mobilization) and insufficient protein supply (by liver synthesis) at the LP formation, may give rise to these triglyceride-loaded VLD-LP, Sf 100—5000. These LP cannot be converted by triglyceride removal alone, according to the assumption in Chapter VII, but need protein addition or polymerization. The vulnerability of the β -LP protein (Paper II), however, speaks against the possibility of this protein moiety occurring in a lipid-free or lipid-poor form able to serve a polymerizing function.

The fact that both apolipoprotein A and C are found within these fractions, Sf 100—5000, makes it a possibility that any of them may participate in the VLD-LP conversion. The evidence for apolipoprotein A as an active factor in the metabolism of triglyceride-rich LP was emphasized in Chapter VII. The qualifications for apolipoprotein C to participate in LP metabolism will be discussed in the next chapter.

CHAPTER IX

The third protein moiety, apolipoprotein C

In the present study on serum chylomicrons and VLD-LP, the protein moieties have been the major interest. Electrophoresis of lipid-rich LP (Figure 2, Paper I) did not necessarily reveal the true mobility of the protein moieties, but showed a pattern obscured by the influence of lipids.

Immunoelectrophoresis, utilizing specific anti-LP sera, showed the presence

in VLD-LP of only one protein moiety, the β -LP protein (55, 135, 136).

Not until experiments on the degradation of chylomicrons and VLD-LP (56, 137, 138) were performed, was evidence for a second, "hidden", protein moiety disclosed. Alpha-LP protein was identified by immunochemical studies in ether extracted chylomicrons (56) and recently also in VLD-LP (138).

In disagreement with these results, studies by N terminal amino acid analysis indicated the presence of as many as four different protein moieties. Thus in addition to N terminal aspartic — and glutamic acid contributed by the α and β LP proteins, respectively, N terminal serine and threonine were also detected (86, 134, 139, 140).

In the present study, a protein moiety contributing N terminal serine and threonine was isolated for the first time^{a)}. Recovered after mild delipidization of VLD LP or chylomicrons, the third protein moiety could be visualized in the analytical ultracentrifuge as a sedimenting phospholipid containing residue, the 7S residue (Chapter V). By using specific antibodies to VLD LP and to whole serum, one characteristic precipitin reaction was obtained in immunoelectrophoresis and diffusion (Chapter V). In view of the fact that the 7S residue (and its protein moiety, the apolipoprotein C) has not been described before, some additional evidence for its actual existence will be taken up.

- a) Randomly adsorbed protein impurities would be eliminated by the purification in the isolation procedure (Chapter I). The high phospholipid content of the 7S residue furthermore excluded the possibility of a serum protein contaminant.
- b) The 4S and the 14S residues, representing previously recognized protein

moieties, α and β LP proteins respectively, may theoretically give rise to products of any physical characteristics. Differences in size, the 7S being the largest (Chapter V), apparently exclude the possibility of this residue being a degradation product of the 4S and the 14S residues. The 7S residue was also studied for the presence of polymerization products by the addition of urea and an anionic surfactant (sodium dodecyl sulfate). Urea in concentrations between 1 and 4 M or the surfactant agent in a concentration of 0.10%, produced no demonstrable alterations in ultracentrifugal characteristics as it would if the 7S residue had been a polymer.

c) Prolonged and repeated lipid extractions with heptane were carried out on fractions containing the 7S residue (Chapter V). These experiments excluded the possibility of the 7S residue being the results of a defect in the delipidization procedure.

In order to visualize the differences (and similarities) in the physical characteristics between the 7S, 4S and 14S residues, a graph was constructed (Figure G) showing a plot of sedimentation coefficient ($S \times \eta$) versus hydrated density (ρ) in the same manner as used in the extrapolation of hydrated density (see Paper V). The similarity in sedimentation coefficients of the 7S residue, the Hdl-2 and the 4S residue within certain solvent densities 1.03–1.07 g/cc, may explain the inability to detect the 7S residue in the analytical ultracentrifuge in earlier experiments with degraded VLD-LP. The slopes of the chylomicrons, $Sf > 5000$, the VLD LP fraction E, $Sf 30$,

^{a)} The question as to whether the presence of two N terminal amino acids indicated two different proteins or two peptide chains linked together cannot be fully settled at this time. However, up until now the immunological studies have shown evidence for only one antigenic protein moiety (Chapter V).

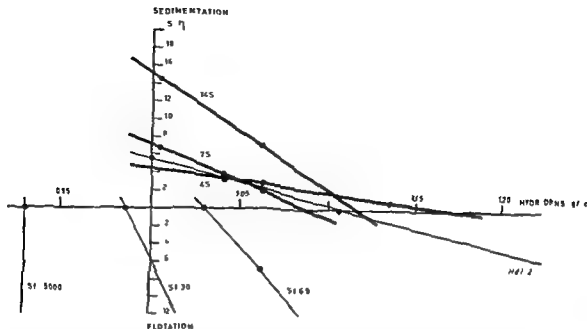


Figure G Sedimentation coefficient ($S \times \eta$) and flotation rate ($Sf \times \eta$) versus solution density (ρ) of the three residues the 4S, the 7S and the 14S, and of four LP fractions the chylomicrons ($Sf > 5000$), the VLD LP fraction E ($Sf 30$), the β LP ($Sf 6.9$) and the α LP ($Hd1.2$). The point at which each line meets the abscissa gives the respective hydrated densities of the residues and the LP. Data from the present study, except for $Sf 6.9$ (50) and $Hd1.2$ (87).

and the β -LP, $Sf 6.9$, are also given in this figure for comparison.

The probability of apolipoprotein C being present as a phospholipid complex in the serum, i.e. in a native apolipoprotein form outside the VLD-LP, has not been possible to confirm in the present study. Some narrow, not completely characterized IP fractions with an electrophoretic mobility of $\alpha-2$ (similar to that of the 7S residue) have previously been described by Shore and Shore (134) in $Sf 0-20$, by Lewis et al (141) in D 10631-1 10 and by Oncley (50) in D 105. Investigations are now under way to test these fractions for the presence of apolipoprotein C.

Two conclusions from the hypothetical estimations on the chylomicron and VLD-LP structure (Chapter VI)

and metabolism (Chapters VII and VIII) gave some suggestions for a possible function for the apolipoprotein C or its phospholipid-protein complex.

Computations of the LP structure suggested that VLD LP, in contrast to β -LP, lacked enough protein-phospholipid-free cholesterol material for an ideal monomolecular distribution in the LP membrane. A "loose" membrane was suggested for these LP, leading possibly to a structural instability, with tendency for disruption and loss of neutral lipids, particularly cholesterol ester and triglyceride (Chapter I). The apolipoprotein C, with a high phospholipid-binding capacity may, in the form of a phospholipid-protein complex, be equipped to serve as a stabilizer for these LP.

Secondly, in the hypothetical conversion of chylomicrons and VLD LP into LP of higher density, the chylomicrons appeared to have sufficient amounts of polymerizing protein in all situations, except possibly in α - α lipoproteinemia. On the other hand, when applied to the triglyceride loaded VLD LP of $S_f > 100$ the conversion theory does not fit, due to a definite lack of circulating apolipoprotein B. The apolipoprotein C, being stable in phospholipid-rich complexes, and possibly present as such a complex in the serum may be able to serve as a substitute for the

apolipoprotein B in the metabolism of these VLD LP.

Only limited information is available at present on this third LP protein moiety and its phospholipid-protein complex. It is speculated, however, that it may participate with α and β LP in the structure and metabolism of the most triglyceride rich VLD LP and possibly the chylomicrons. It should be stressed at this point, however, that no other metabolic system in the body is known to be supplied with such a substituting factor.

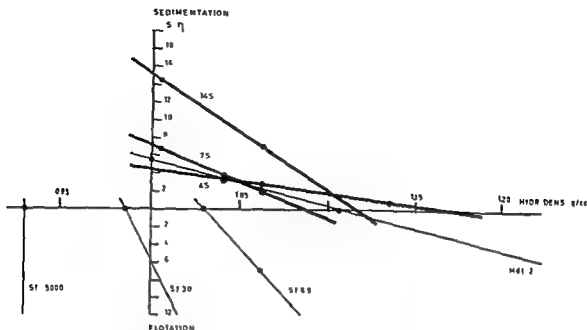


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characteristic phospholipid protein residues with approximate sedimentation coefficients 4S, 14S and 7S. Partially delipidized VLD-LP, $S_f > 20$ contained all three residues. The distribution of the three residues among chylomicrons and VLD LP showed that the chylomicrons contained mainly the 4S residue and that the 14S residue was present in VLD LP. The 7S residue was found within chylomicrons as well as VLD-LP.

The three residues were separated and each characterized by its protein moiety. The 4S contained α -LP protein, apolipoprotein A, the 14S contained the β LP protein, apolipoprotein B, and the 7S, finally, appeared to contain a third protein moiety not previously isolated, apolipoprotein C. This latter protein gave as characteristic N terminal amino acids serine and threonine, showed one precipitin band with antibodies to human serum and human serum VLD LP, and had peptide patterns different from those of the other two LP proteins.

Chapter VI A possible model of the micellar LP, is discussed. Physical characterization and chemical composition of the β LP are consistent with a monomolecular membrane containing an association of free cholesterol, phospholipid and protein surrounding a lipid core composed of cholesterol ester and triglyceride. The composition of the VLD LP was insufficient for an ideal monomolecular layer covering their entire LP surface. A loose membrane of similar appearance was suggested for these LP. This loose membrane could

explain the experimentally observed instability of the VLD-LP structure. It was also estimated that enough space was available to accommodate for the cholesterol ester and triglyceride molecules (in liquid distribution) within the central sphere of all VLD and β -LP.

Chapter VII A hypothetical intravascular metabolism of chylomicrons by their conversion into VLD LP and ultimately into α -LP was computed from the LP data. This conversion assumed, besides triglyceride removal, also protein addition, possibly as a polymerization of α LP protein. The computations gave intermediate conversion products with a composition remarkably similar to that of the experimental fractions.

Chapter VIII The metabolism of VLD-LP by their conversion into β -LP was supported by estimations of the mole lipid composition of VLD and β -LP. Conversion by intravascular hydrolysis of triglyceride appear applicable in the normally occurring VLD LP, $S_f 20-100$. On the other hand, in less dense VLD-LP, $S_f > 100$ present in excess in hyperlipemic states, the metabolism may go through a different mechanism as judged from these estimations and known properties of the β LP protein.

Chapter IX The third LP protein, apolipoprotein C, or its phospholipid protein complex, may serve a function in the transformation into LP of higher density of these less dense VLD-LP, $S_f > 100$ and possibly under certain conditions also of the chylomicrons.

Summary

Chapter I A standardized procedure for the ultracentrifugal separation and purification of chylomicron and VLD-LP fractions was developed. With this method any number of narrow sub-fractions of these LP can be isolated. Five arbitrarily chosen fractions were isolated and characterized. Chemical and physical data of these LP indicated that the chylomicrons and the VLD-LP contained a continuous spectrum of heterogeneous LP of decreasing size and increasing density. Judged by the greater loss of certain lipids by the isolation-purification procedure, particularly cholesterol ester, a certain instability of their structure was suggested.

Chapter II The protein moieties of the chylomicrons and the VLD-LP were studied to verify whether differences in these might be the cause of the LP heterogeneity. Lipid-free protein moieties of the chylomicrons and the VLD-LP could not be recovered in water-soluble form. A method for partial delipidization, particularly applicable to the lipid-rich VLD-LP, was developed. Repeated heptane extractions of LP that were lyophilized in the presence of insoluble starch gave reproducible, neutral lipid-free phospholipid-protein residues of all serum LP.

Certain differences in the extractibility of the lipids were observed.

Chapter III Three different dietary types of hyperlipemia were each characterized by the presence in excess of certain LP fractions. Subjects presenting evidence for fat-induced hyperlipemia, (type I) (67), carbohydrate-induced hyperlipemia, (type IV), and a mixed dietary form, (type V), were studied in a limited number of cases and compared with the VLD-LP distribution in the normal subject. The type I hyperlipemia was characterized by large amounts of chylomicrons, the type IV of cholesterol and triglyceride-rich VLD-LP, Sf 100—400, and finally type V, showed the presence in excess of "atypical chylomicrons" as well as VLD-LP, Sf 100—400.

Chapter IV Chylomicrons were difficult to separate and characterize, particularly in the presence of excessive amounts of VLD-LP as in most types of hyperlipemia. It is suggested that their characterization by protein moiety may be useful.

Chyle chylomicrons and serum chylomicrons showed certain differences in lipid-protein composition. These differences were taken into consideration in the discussion of their metabolism (Chapter VII).

Chapter V The method of partial delipidization of the serum LP gave three

great share in this work. Without her support and encouragement this thesis would never have been completed.

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SUPPLEMENTUM 44

ASYMMETRY OF RENAL FUNCTION WITH SPECIAL REFERENCE TO CHRONIC PYELONEPHRITIS

by

PER ÅKE ÖRSTEN

Accompanied by 180

STOCKHOLM 1966

From the IVth Medical Clinic (Renal Clinic) and the Clinical Central Laboratory
S:t Eriks sjukhus Stockholm Sweden

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ERRATA

Page 18 column 1 line 2 from bottom — for sodium, $\frac{U_{Na}}{P_{Na}}$ — read creatinine $\frac{U_{Cr}}{P_{Cr}}$

Page 31 column 2 line 7 — for Raascrou — read Raaschou

Page 36 column 2 line 2 from bottom — for patent — read patient

Page 43 column 2 line 5 — for 00—00 — read 112—113

Page 45 column 1 line 14 — for Na $\frac{U_{Na}}{P_{Na}}$ — read Cr $\frac{U_{Cr}}{P_{Cr}}$

Page 48 column 1 line 5 — for VIII — read VII

Page 51 column 2 line 7 from bottom — for rise — read fall

Page 71 75 77 81 Table Xa Xb column 5 — for U_{Cr} (mEq/lit) — read U_{Cr} (mg/100 ml)

Page 90 column 1 line 10 from bottom — for 2B — read 2A

Page 105 column 1 line 7 — for ability;PAH — read ability PAH

Page 105 column 1 line 14 — for urine — read blood

Page 106 column 1 line 17 from bottom — for 55 — read 8

Page 115 column 2 line 9 and 14 from bottom — for Na — read K

Page 121 column 1 line 11 — for Departement — read Department

To my wife and children

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Translated from the Swedish

by

ERICA ODELBORG

INTRODUCTION

It is an established fact that in persons with healthy kidneys renal function is as a rule practically symmetric (Table I). This seems to be an expression of the symmetric structure of the human body. Nevertheless even if the body has a symmetric structure in many respects there are several examples of asymmetry between paired organs e. g. the right and left hemispheres of the brain, and the right and left lung.

A priori it could be expected that those renal diseases which involve the whole organ more or less homogeneously would not necessarily alter the symmetry of renal function. On the other hand it seems plausible to assume that those renal diseases which are obviously unilateral — e. g. a tumour, stricture of an artery, malformation of the urinary tract or cyst — would give rise to marked asymmetry. Between these two extremes we have types of renal damage in which it is questionable whether or not we can expect the symmetry of function to be affected. Foremost among these diseases is pyelonephritis in which experience has shown that histologically the degree of damage may differ on the two sides irrespective of the pathogenesis.

Both acute and chronic pyelonephritis are caused by pathogenic organisms, which invade the kidney in varying number. Consequently in the initial stage of pyelonephritis there are always a variable number of foci of infection. It seems unlikely that their spreading size and increase in number would invariably or most frequently be

such that the renal damage is homogeneous and the same in both kidneys.

Several workers have compared the function of the right and left kidney in both acute and chronic pyelonephritis as well as in preponderantly unilateral renal diseases. Interest in the diagnosis of renal artery stenosis as a cause of arterial hypertension has widened the indications for split function tests of the kidneys. A survey of previous studies on the function of each kidney separately in patients with acute and chronic pyelonephritis is given in Table II which does not however have any pretensions to completeness. A considerable number of such split function tests in other renal diseases have also been described.

It is evident from this table that the asymmetry in pyelonephritis has been studied by means of several parameters of renal function. I have however been unable to trace any investigation in a large series in which functional tests have been made at both ureteric and renal vein catheterization.

In the present investigation my main interest was focused on a comparison between the symmetry of renal extraction of para aminohippuric acid (PAH) and the symmetry or asymmetry of the renal concentration ability and the ureteric urine's content of electrolytes and bacteria. In certain cases other parameters of renal function were also investigated in this respect. At the Renal Clinic and the Clinical Central Laboratory, St. Erik's Sjukhus, these investigations among others have been made in a

IX RESULTS	69	D Clinical Value of Testing for Asymmetry of Renal Function	110
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INTRODUCTION

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Table II Some previous reports on the symmetry of renal function in patients with acute and chronic pyelonephritis

Author	Year	Type of investigation										
		Uosmol	Diuresis	Ulelectrolytes	Uosm UNIT	Dye tests	EPH ED	Clearances	RPF RBF Tm	Roentgenologic exam	Radioactive renogram	Other determinations
Bergström <i>et al</i> (15)	1959						x					
Bricker <i>et al</i> (27)	1958	x				x		x	x			
Bumpus (46)	1931	x										
Dejdar (62)	1959									x	x	
Dejdar & Prat (63)	1958									x	x	
Dustan <i>et al</i> (64)	1958		x	x								
Edvall (69)	1958						x	x	x			
Fullerton (82)	1923	x	x									
Göthgen (86)	1936							x				
Hellström (95)	1931					x						
Hent (96)	1941				x							
Hradkova & Schuck (107)	1958		x	x								
Kjellbo <i>et al</i> (134)	1962	x	x	x	x			x	x			
Kusunoki <i>et al</i> (143)	1956						x					x
Michie & Michie (163)	1957							x	x			
Minder (166 167)	1929	x										x
Ockerblad (178 179)	1925											x
Ofstad (181)	1965						x					x
Olsson (182)	1962									x		
Örsten (185)	1962						x					
Parkman (189)	1965						x					
Poutasse <i>et al</i> (192)	1958		x	x				x		x		
Prat <i>et al</i> (195)	1958	x						x				
Richardson <i>et al</i> (210)	1965	x	x	x	x			x	x	x		
Rothauge (214)	1957							x				
Rowntree & Geraghty (216 217)	1910					x						
Saheki (219)	1912					x						
Schück & Hradec (223)	1929	x				x						x
Schück <i>et al</i> (224)	1958		x	x								
Todd (249)	1960		x	x								
Todd (249)	1929	x				x						
Veres <i>et al</i> (259)	1964		x	x						x		
Vuust (262)	1936							x				x
Winter (270)	1956										x	

of patients with the relevant diseases at the Renal Clinic the size of the present series is limited. This is chiefly because both bilateral renal vein catheterization and bilateral ureteric catheterization could not be carried out in every case.

The series includes only patients who were hospitalized at the Renal Clinic, and

who underwent thorough investigation there. At this clinic both renal vein and ureteric catheterization are performed as routine clinical examinations in collaboration with the Clinical Central Laboratory and the Departments of Urology and Roentgenology.

Initially the object of the investigation

Table I *Some previous reports on the symmetry of renal function in healthy men*

Author	Year	Type of investigation	No of invest	Symmetric function no
Boyd & Murdock (24)	1962	Radioactive renogram	10	10
Bumpus (46)	1931	Phenolphthalein app test + specific gravity	70	56
Fullerton (82)	1923	Specific gravity	117	111
Haugen <i>et al</i> (94)	1960	C _{In} + C _{Cr} + C _{Urea} + CPAH	14	14
Henl (96)	1941	UNH ₂	17	13
Hradkova & Schück (107)	1958	Conc index $\frac{U_{Na}}{P_{Na}}$	8	8
Hulet <i>et al</i> (108)	1960	U _{Na}	17	13
		U _{Osmol}	17	14
		C _{In}	21	20
		CPAH	17	16
		Diuresis	21	18
Magnusson (158)	1962	Radioactive renogram	5	5
Michie & Michie (162)	1951	C _{In} + CPAH	140	98
Ofstad (181)	1965	EPAH	4	3
Prat <i>et al</i> (195)	1958	Conc index $\left(\frac{U_{Cr}}{P_{Cr}} \frac{U_{In}}{P_{In}} \right)$	16	16
Prat & Kocvara (197)	1957	Conc index $\left(\frac{U_{Cr}}{P_{Cr}} \right)$	8	6
Rowntree & Geraghty (216)	1910	Phenolphthalein app test	7	7
Sack (218)	1940	UNH ₂ + indigo carmine app test	5	5
Saheki (219)	1929	U _{Cr}	12	10
		U _{Osmol}	10	10
		Phenolphthalein app test	13	11
Schuck <i>et al</i> (224)	1960	U _{Na}	14	9
		U _{Cr}	14	9
Todd & Crowell (250)	1925	Phenolphthalein app test + specific gravity	31	28
Torrance <i>et al</i> (251)	1961	Radioactive renogram	19	19
Wedeen <i>et al</i> (266)	1963	Radioactive renogram	37	37
Winter (270)	1956	Radioactive renogram	5	5

large series of patients with renal diseases, especially chronic pyelonephritis. Consequently, I considered that a survey of the results as regards the symmetry (or asymmetry) of renal function would be of interest as a comparison and complement to other clinical roentgenologic and anamnestic data.

The diagnosis of chronic pyelonephritis is often difficult, particularly when the disease is in a latent stage. I therefore regarded it as worth while to ascertain whether the results obtained could provide information of pathophysiologic interest and

whether they could be of diagnostic value. The present thesis comprises a collation of these results. I have included as comparative material some cases of other diseases affecting the kidneys such as essential hypertension, stenosis of the main renal artery with hypertension, chronic glomerulonephritis, the nephrotic syndrome and unilateral urologic diseases. In many cases both renal vein and ureteric catheterization were performed but in some patients only the latter examination was made or *vice versa*. Even if we have a fairly large material

CHAPTER I

CRITERIA OF ASYMMETRY

No sharp borderline can be drawn for what is to be denoted as asymmetry with respect to renal function. Strictly speaking asymmetry is present when the difference between the function of the two kidneys is greater than the spontaneous range of variation in each separate kidney. One can however scarcely expect the function to be completely symmetrical since it is known that differences normally exist between the kidneys as regards such factors as size, vascular bed and thickness of parenchyma. This implies that in order to evaluate pathologic asymmetry a study should be made of the extent to which asymmetry exists in healthy individuals. On ethical grounds such a normal series is impracticable when analyses are to be made of urine obtained by ureteric catheterization. Consequently I have been obliged to evaluate my results in this respect by comparisons with those of other workers.

The most extensive investigation of separate renal function tests in persons without demonstrable renal disease was reported by Hulet *et al* (109). Their observations were made in 21 normotensive patients (19 women and 2 men) hospitalized at a medical clinic. A few values in their tables suggest that renal disease cannot be ruled out with certainty in every case although these divergent values were ascribed by the authors to technical errors. The difference between the kidneys with respect to sodium concentration in urine, diuresis, osmolality, paraaminohippuric acid and inulin clearance was calculated as a percentage of the individual

mean value. Since the frequency curve of these differences showed a skew distribution special statistical methods had to be used for calculating the normal values. Thus the difference between right and left kidney was calculated by dividing the difference Δ by the mean of the values for both kidneys. This gave a percentage difference in relation to the mean value:

$$\begin{aligned}\text{percentage difference} &= \frac{\Delta}{\frac{R+L}{2}} \times 100 \\ \text{or} \\ \frac{2\Delta}{R+L} \times 100 \quad (84)\end{aligned}$$

According to Hulet *et al* the mean values of the various parameters of function were higher for the left kidney than for the right. The authors did not however present any statistical evidence for predominance of one kidney. As far as one can judge from the values published they do not show any significant preponderance of the left kidney.

In 5 patients in my series bilateral renal function tests were made for suspected renal disease but on collation of the results no pathologic processes were demonstrable in the kidneys. Despite this I was unable to regard them as definitely healthy in this respect in view of previous symptoms from the kidneys or lower urinary tract. Consequently I have no personal normal series of subjects who had undergone ureteric catheterization. On the other hand as far as analyses of renal vein catheterization are concerned I have a small series as comparative

was not only to study the conditions of symmetry and diagnosis but also to study the effect on renal function of treatment of chronic pyelonephritis — particularly long term therapy — as described in several papers from the Renal Clinic and the Clinical Laboratory (42, 44, 45, 104, 183-184, 186). However, in view of the lack of beds

at the Hospital, the latter task could not be carried out to a sufficient extent to merit a detailed report (*cf* Chap. X, pp. 112-113). Nevertheless, it is my hope that the present material will provide an adequate basis for subsequent evaluation of the influence of treatment on renal function, among other matters.

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CRITERIA OF ASYMMETRY

No sharp borderline can be drawn for what is to be denoted as asymmetry with respect to renal function. Strictly speaking asymmetry is present when the difference between the function of the two kidneys is greater than the spontaneous range of variation in each separate kidney. One can however scarcely expect the function to be completely symmetric, since it is known that differences normally exist between the kidneys as regards such factors as size, vascular bed, and thickness of parenchyma. This implies that in order to evaluate pathologic asymmetry a study should be made of the extent to which asymmetry exists in healthy individuals. On ethical grounds such a normal series is impracticable when analyses are to be made of urine obtained by ureteric catheterization. Consequently, I have been obliged to evaluate my results in this respect by comparisons with those of other workers.

The most extensive investigation of separate renal function tests in persons without demonstrable renal disease was reported by Hulet *et al.* (108). Their observations were made in 21 normotensive patients (19 women and 2 men) hospitalized at a medical clinic. A few values in their tables suggest that renal disease cannot be ruled out with certainty in every case, although these divergent values were ascribed by the authors to technical errors. The difference between the kidneys with respect to sodium concentration in urine, diuresis, osmolality, paraaminohippuric acid and inulin clearance was calculated as a percentage of the individual

mean value. Since the frequency curve of these differences showed a skew distribution special statistical methods had to be used for calculating the normal values. Thus the difference between right and left kidney was calculated by dividing the difference Δ by the mean of the values for both kidneys. This gave a percentage difference in relation to this mean value:

$$\text{percentage difference} = \frac{\Delta}{\frac{R + L}{2}} \times 100$$

or

$$\frac{2 \Delta}{R + L} \times 100 \quad (81)$$

According to Hulet *et al.* the mean values of the various parameters of function were higher for the left kidney than for the right. The authors did not however present any statistical evidence for predominance of one kidney. As far as one can judge from the values published they do not show any significant preponderance of the left kidney.

In 5 patients in my series bilateral renal function tests were made for suspected renal disease but on collocation of the results no pathologic processes were demonstrable in the kidneys. Despite this I was unable to regard them as definitely healthy in this respect in view of previous symptoms from the kidneys or lower urinary tract. Consequently I have no personal normal series of subjects who had undergone ureteric catheterization. On the other hand, as far as analyses in renal vein catheterization are concerned I have a small series as comparative

was not only to study the conditions of symmetry and diagnosis but also to study the effect on renal function of treatment of chronic pyelonephritis — particularly long term therapy — as described in several papers from the Renal Clinic and the Clinical Laboratory (42, 44, 45, 104, 183, 184, 186) However, in view of the lack of beds

at the Hospital the latter task could not be carried out to a sufficient extent to merit a detailed report (*cf* Chap X, pp 112—113) Nevertheless, it is my hope that the present material will provide an adequate basis for subsequent evaluation of the influence of treatment on renal function, among other matters

disease. However a number of workers have shown (cf p 32) that asymptomatic bacteriuria occurs in up to 30 per cent of patients on admission (147) and that demonstrable chronic pyelonephritis develops in e.g. 35—40 per cent of women with asymptomatic bacteriuria during pregnancy (129). Consequently such cases cannot be ruled out with certainty in the series stated to consist of patients with healthy kidneys.

In the present series the functional tests whose results were classified as denoting symmetry or asymmetry respectively were always made before instituting any therapy. If the patient was taking any drug when admitted, this medication was discontinued for a week or two before the tests.

Difference in Concentration Ability

Bilateral determination of the specific gravity was reported in 1923 by Fullerton (82). Such comparisons have subsequently been made in connexion with bilateral studies of the excretion of phenol red after injection of the dye (46, 250). The results of these three investigations are summarized in Table I. The authors gave no details of their material nor can it be inferred whether the left or the right kidney had poorer function.

Among the studies made by Saheki (219) in 7 patients with healthy kidneys was concurrent determination of the specific gravity of the urine from the right and left kidney. He found bilateral agreement in every case (range of variation 0.000—0.001). This range of variation was also noted in three patients with movable kidneys. The test was evidently made in well hydrated subjects since the specific gravity ranged from 1.001—1.010 except in two cases (sp. gr. 1.026—

1.025 and 1.025—1.025 on the right and left side respectively). Bumpus (46) reported that only 19 of 70 patients regarded as having healthy kidneys had identical specific gravity in the urine from both sides. If on the other hand, the limit of the difference was set at 0.00256 (80 per cent) of the 70 had the same specific gravity. In the remaining 14 cases the difference ranged from 0.003 to 0.014. Since the data in the tables are confined to these 14 cases there is no possibility of calculating the percentage differences. Nor is it evident whether the right or left kidney had poorer function when a discrepancy was observed. Hulet *et al.* (108) made simultaneous determinations of the osmolality of the urine in 17 patients whose kidneys were evaluated as completely healthy. In 14 of them the difference between the urine from the right and left side ranged from 0.12 to 7.36 per cent. In three cases the difference was 16.1, 17.1 and 27.0 per cent respectively. The mean value of the difference was 3.81 per cent but if the three greatly divergent values are omitted the corresponding figure is 2.76 per cent. Hulet *et al.* stated that they were able to show an inappreciable dominance of the left kidney (see Chap. II).

In view of the aforementioned observations and of personal experience I have considered it justified to set the borderline between symmetry and asymmetry at a difference of 10 per cent in osmolality.

Difference in Urinary Creatinine Concentration

A comparison between the creatinine concentration in the urine of both kidneys was made by Schüick *et al.* (224) in 8 subjects considered to have healthy kidneys.

Table III PAH extraction ratio in 11 healthy volunteers

Subject no	EPAH Right Kidney	EPAH Left Kidney
1	92.4	90.0
	91.6	91.0
	93.7	90.6
		94.7
		95.6
2		94.3
	92.8	92.5
	92.1	92.8
	92.4	92.7
3	88.9	88.2
	90.3	89.0
	90.0	89.5
4	89.5	91.8
	89.3	90.4
	89.1	87.8
5	92.0	92.2
	92.3	92.4
	93.4	92.3
6	93.7	94.1
	94.3	95.0
	93.2	93.9
7	89.8	90.7
	90.4	89.8
	91.3	89.3
8	93.3	90.6
	86.3	91.4
	84.7	90.7
9	90.1	88.3
	86.1	89.3
	88.2	87.9
		88.5
		90.7
		90.7
		90.6
10	91.3	91.3
	90.3	92.0
	92.0	91.9
11	90.0	90.9
	89.1	90.9
	89.3	90.6

EPAH mean right kidney 90.74 %
EPAH mean left kidney 91.13 %

material consisting of 11 healthy volunteers (Table III)

In the following, the difference between right and left kidney as regards the various parameters of function is expressed — as done by Hulet *et al* — as the percentage difference and not in absolute figures. This is because when using a percentage difference one can, *e.g.* compare the kidneys with respect to various functional differences at various absolute levels. Moreover, this method permits comparisons between the renal function on different occasions in the same patient, as well as comparisons between the results in different patients. Difficulties arise if the comparison is based on the relation between the values for one kidney and the other, *e.g.* right:left (5). Thus with a given difference between the two kidneys, the relation will vary, depending on whether the percentage value is calculated on the function of the better or the poorer kidney.

On the basis of these results, I have set up the criteria described in the following for renal function to be regarded as asymmetric: It must however, be emphasized that these criteria are somewhat arbitrarily chosen since they are based on small series reported by several workers. These investigations were, as a rule made in patients hospitalized for some other illness than renal

Three extraction values were first determined on the right side followed by 3 or more on the left. The individual means were calculated only from the first 3 values for each kidney.

Difference right left kidney

$$d = -0.23 \pm 0.35$$

$t = 0.65$ No significant deviation from 0

Difference right left kidney in the same subject

$$|d| = 0.90 \quad s = 0.70$$

95 % limits with 95 % confidence ($n = 11$)

$$0.90 \pm 3.26 \cdot 0.70 = +3.18 - -1.39$$

Standard deviation of the values in the kidneys of the individual

Right $s = 1.94$

Left $s = 0.77$

disease. However a number of workers have shown (cf p 32) that asymptomatic bacteriuria occurs in up to 30 per cent of patients on admission (147) and that demonstrable chronic pyelonephritis develops in e.g. 33—40 per cent of women with asymptomatic bacteriuria during pregnancy (129). Consequently such cases cannot be ruled out with certainty in the series stated to consist of patients with healthy kidneys.

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1.025 and 1.025—1.025 on the right and left side respectively). Bumpus (46) reported that only 19 of 70 patients regarded as having healthy kidneys had identical specific gravity in the urine from both sides. If on the other hand the limit of the difference was set at 0.00256 (80 per cent) of the 70 had the same specific gravity. In the remaining 14 cases the difference ranged from 0.003 to 0.014. Since the data in the tables are confined to these 14 cases there is no possibility of calculating the percentage differences. Nor is it evident whether the right or left kidney had poorer function when a discrepancy was observed. Hulet *et al.* (108) made simultaneous determinations of the osmolality of the urine in 17 patients whose kidneys were evaluated as completely healthy. In 14 of them the difference between the urine from the right and left side ranged from 0.12 to 7.36 per cent. In three cases the difference was 16.1, 17.1 and 27.0 per cent respectively. The mean value of the difference was 3.81 per cent, but if the three greatly divergent values are omitted the corresponding figure is 2.76 per cent. Hulet *et al.* stated that they were able to show an inappreciable dominance of the left kidney (see Chap. II).

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A comparison between the creatinine concentration in the urine of both kidneys was made by Schück *et al.* (224) in 8 subjects considered to have healthy kidneys.

Great differences were found between the two sides as regards creatinine concentration the mean value of the difference in 14 paired determinations being 21.10 per cent (range 6.06—53.66). The authors attributed this wide scattering to osmotic diuresis caused by the procedure of examination and not to any pathologic process in the kidneys.

Better agreement between the kidneys was observed by Saheli (219) in an investigation of 12 persons with healthy kidneys. He stated that the mean value of the difference between the creatinine concentration in the urine from either side was 9.10 per cent (range 0—22.22).

Chiefly on the basis of Saheli's results I have drawn this borderline at a difference of 20 per cent between the kidneys.

Difference in Urinary Sodium Concentration

To investigate the symmetry of the urinary sodium concentration Schuck *et al* (224) made 14 bilateral determinations in 8 subjects with healthy kidneys and lower urinary tract. They noted a mean difference of 10.43 per cent (range 1.34—22.22). Hulet *et al* (108) found a corresponding mean value of 6.99 per cent in their series of 17 healthy subjects. Four values nevertheless deviated greatly from the others *viz* 10.0, 15.5, 16.0 and 26.7 per cent. The mean difference when these were omitted was 3.89 per cent (range 1.48—8.80).

In view of the observations of Hulet *et al* I have somewhat arbitrarily set the limit of symmetry at a difference of 10 per cent. However taking into account some previous reports one must anticipate occasional greater asymmetry even in subjects with healthy kidneys.

Difference in Urinary Potassium Concentration

I have been unable to find any data in the literature regarding the symmetry of this parameter of renal function in persons with healthy kidneys. In analogy with the osmolality and sodium concentration the borderline has therefore been drawn arbitrarily at a difference of 10 per cent.

Difference in Diuresis

In the series of Hulet *et al* (108) the mean difference between the diuresis of the two kidneys in 21 subjects with intact renal function was given as 5.17 per cent (range 0.00—26.3). When the three most deviating values (26.3, 15.2 and 10.3) were excluded the mean value was 3.16 per cent (range 0.00—8.17). In my series the diuresis of the two kidneys separately was determined only in patients with renal artery stenosis with arterial hypertension in addition to two patients with essential hypertension. The borderline for asymmetry was therefore drawn at a difference of 50 per cent between the kidneys in accordance with the values of several authors (56, 57, 106, 220, 276).

Difference in pH of Urine

The ammonia and titratable acid content of bladder and ureteric urine was determined in many cases in the present series (*cf* Chap VII). Since for various reasons the determinations were not made consistently these values have not been listed in the tables. The pH was also determined but this test on the ureteric urine was not as a rule preceded by acidification partly because the patient was deprived of fluids until the urinary concentration test was made concurrently.

The pH was therefore regarded to be of inappreciable value as a basis for evaluating the acidifying ability of one kidney in relation to that of the other. The total acidifying ability of the kidneys was on the other hand determined after an ammonium chloride load.

Sack (218) stated that the ammonia concentration was identical in the urine from both kidneys and Henri (96) demonstrated that the difference did not exceed 10 per cent in subjects with healthy kidneys. As far as I have been able to ascertain, the literature contains no corresponding data on the pH of the urine. However, since the error of the method is exceedingly small, I have set the borderline for the difference in pH at 0.05. Obviously this difference cannot be calculated as a percentage.

Difference in Clearances

Separate *endogenous creatinine clearances* were calculated in clinically healthy patients by e.g. Haugen *et al* (94) in their 14 cases; they found good agreement between the kidneys in this respect. This also applied to a patient with chronic glomerulonephritis. The greatest difference noted was 5 ml/min corresponding to 6 per cent.

Haugen *et al* (94) also determined the *inulin* and *para aminohippuric acid (PAH)* clearance in the ureteric urine of the same 14 subjects. Here as well there was good agreement with no difference between the kidneys for inulin and a mean difference of 2.5 per cent for PAH.

Hulet *et al* (108) made 2% C_{in} determinations in the urine of each kidney in their aforementioned series. The mean of the differences between the kidneys was 7.59 per cent (range 0.37—21.2). In 17

determinations of CPAH the corresponding mean was 6.34 per cent (range 0.32—20.2).

Michie & Michie (162) also compared these two parameters in 110 determinations (number of subjects not stated). In more than 70 per cent, they found that the inulin and PAH clearance was almost identical for both kidneys. They did not present any tables or data regarding the results in the remaining 30 per cent of the determinations. In a study of patients with renal tuberculosis Hørg (102) expressed the view that the sources of error in determining the urinary clearance of urea, inulin, endogenous creatinine and PAH do not permit greater accuracy than ± 5 per cent.

Since I have no series of healthy subjects, I have relied entirely on the aforementioned data with respect to clearance determinations as well. I have set the borderline for asymmetry at a difference of 20 per cent between the kidneys.

Difference in Renal Plasma Flow

I have not succeeded in tracing any reports of studies on the difference in renal plasma flow (RPF) between the two kidneys of healthy subjects. Consequently, in analogy to the differences in clearance, the borderline has been fixed at 20 per cent.

Difference in

Para Aminohippuric Acid Extraction

The literature contains a number of data on normal values for the total para aminohippuric acid (PAH) extraction (14, 23, 33, 47, 143, 208, 232, 265). As a rule this has been calculated on the right kidney. Bucht (41) reported the results of 29 renal vein catheterizations made on the right kidney with a

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the difference was counted. This grading is in conformity with bacteriologic practice (*cf* Chap. VII).

Difference in Certain Clinical Features

Obviously such clinical features as a feeling of tension, pain in the flank, and tenderness to palpation over one or both

loins are highly subjective criteria. They are however of considerable value not only for the diagnosis of pyelonephritis in general but also for establishing the predominance of the disease on one side or the other. Consequently the existence of a difference between the kidneys in this respect was compared with that of asymmetry in the functional tests.

mean PAH extraction of 87.9 per cent (range 84—96), and 8 tests in other healthy subjects, in which the mean extraction by the left kidney was 87.4 per cent (range 83—91)

Ofstad (181) reported determination of the EPAH by the right and the left kidney in 4 subjects with normal renal function. In 3 cases, the difference ranged from 2.20—3.30 per cent. In the remaining case, the difference between the kidneys was 9.09 per cent, with a mean of 4.24 per cent for all tests. Since, as far as I am aware, no other simultaneous study has been made of the PAH extraction by the two kidneys separately, I made this test in 11 healthy subjects. The mean extraction by the right and the left kidney was 90.7 and 91.1 per cent, respectively (cf Table III). On the basis of these results, I set the borderline for asymmetry between the kidneys with respect to PAH extraction at 3 per cent.

Roentgenologic Examination

Cases have been denoted as roentgenologic ally asymmetric only when unquestionable differences between the kidneys were visible on the films. When evaluating these differences, I have relied exclusively on the statements of the roentgenologist who studied the films of the upper urinary tract of all the patients without knowledge of the other data in these cases.

Evaluation of differences between the kidney and ureter of either side was based largely on the criteria of Dejdar (62) Dejdar & Prat (63) and Olsson (182). On the plain films, the kidneys were compared with respect to size and shape, sharpness or irregularity of the contours, and presence of concretions if any. The excretory

pyelograms were studied to demonstrate differences between the thickness of the renal parenchyma, dilatation of the pyeloureteric system, and deformation of the calyces, e.g. papillary atrophy and destruction, and rounding of the fornices. Delayed or poor excretion of contrast medium and density of the medium were also evaluated.

As comparative material, I included cases of renal disease in which involvement was regarded as homogeneous, and in which roentgenologic examination did not disclose any difference between the two kidneys.

In addition, patients with preponderantly unilateral renal disease were investigated. In some of the latter cases, examination disclosed developmental anomalies, concretions in the renal pelvis, hydronephrosis or hydroureter, sometimes caused by ureteric concretions, stricture or reflux. In this group the difference between the two sides was easier to detect. If functional changes were present — e.g. variations in tonus in the upper urinary tract — and affected the roentgenologic features, they were not taken into account.

Renal angiography was performed in hypertensive patients with suspected stenosis of the main renal artery, as well as in pyelonephritis and other renal diseases, to provide a more all round picture of such features as size of the kidney and the existence of scarring. In addition, retrograde pyelography and micturition cystography were done in some cases partly to illustrate the asymmetric involvement by the pathologic process.

Difference in Bacterial Count

When the number of bacteria in the urine from one kidney was more than 10 times as great as that in the urine from the other

who had died in accidents or by violence and demonstrated that the kidneys decrease in size after 50 years of age

Roentgenologic studies have also been published which confirm the aforementioned anatomic differences between healthy kid-

neys (168 188 222) The most extensive investigation of this kind hitherto reported is that of Moëll (169) who measured the length, width and area of both kidneys in 100 healthy men and 100 healthy women The mean values were as follows

MEN	Right kidney	Left kidney
Length \times width (cm)	12.9 ($s = 0.80$) \times 6.2 ($s = 0.45$)	13.2 ($s = 0.79$) \times 6.3 ($s = 0.49$)
Area (cm ²)	79.6 ($s = 8.75$)	82.7 ($s = 8.34$)
WOMEN		
Length \times width (cm)	12.3 ($s = 0.79$) \times 5.7 ($s = 0.46$)	12.6 ($s = 0.77$) \times 5.9 ($s = 0.42$)
Area (cm ²)	70.1 ($s = 8.00$)	74.1 ($s = 7.31$)

Moëll concluded from these figures that both the right and left kidney are significantly larger in men than in women and that the left is larger than the right in both sexes

As mentioned in Chapter I Hulet *et al* (108) claimed to have found a functional dominance — even if it was inappreciable — of the left kidney this was not however

demonstrated statistically Nor did they demonstrate any sex difference with respect to renal function

The authors who have studied the various functions of both kidneys in healthy subjects and the incidence of asymmetry reported by them are listed in Table I The table has no pretensions to completeness

EARLIER BILATERAL COMPARISONS BETWEEN HEALTHY KIDNEYS

In the previous chapter, I gave an account of the criteria of differences between the two kidneys with respect to the parameters of renal function applied in the present study. In the following, a brief survey is given of some earlier investigations on the symmetry of healthy kidneys as regards both function and anatomy.

As early as 1893, Suter & Meyer (246) reported determinations of the *diuresis* from each kidney in a healthy 5 year old boy. The urine was collected from the ureteric orifices which opened into an ectopic bladder. During a 6 hour period, the excretion of urine, urea and phosphoric acid was approximately the same from both kidneys. The difference in diuresis during the $3\frac{1}{2}$ days duration of the test was 4.5 per cent, the lower value being noted for the left kidney.

It was not until Nitze of Vienna introduced a practicable cystoscope in 1877 (48) that the prerequisites were created for catheterizing the ureters. The first properties to be investigated were the urinary output, specific gravity of the urine and its content of various metabolites and electrolytes (e.g. urea, creatinine and sodium), and the excretion of certain dyes.

The tubular reabsorption of water was studied by Hradkova & Schuck (107) with the help of the *concentration index*

of sodium, $\frac{U_{Na}}{P_{Na}}$. They found no marked

difference in this respect between the two

kidneys of children with normal renal function.

Prat *et al* (195) calculated the *concentration index* of the urine from each kidney and found good bilateral agreement. They defined the concentration index as the ratio of the creatinine or inulin in urine to that in plasma.

Many studies of renal function are based on determinations of the rate of excretion by the kidneys of a dye administered intravenously or orally. As early as 1910, Rowntree & Geraghty (216) found good conformity between the kidneys of healthy subjects with respect to the excretion of *phenol red*.

In recent years *renograms* have been suggested as a general screening test of the symmetry of renal function. They were first described by Winter (270) who stated that identical renograms of both kidneys could be observed when renal function was normal. This was confirmed by later workers (24, 158, 251, 266).

Several authors have described *anatomic* comparisons between the kidneys. Thus in 1872 Pourteyron (191) made a post mortem examination of 86 pairs of kidneys and found that the left was slightly larger in both sexes. Moreover both kidneys were somewhat smaller in women than in men and their total weight was lower. The left kidney has also been found to weigh more than the right (148, 191). De Leon (148) made post mortem studies in 769 persons

teria or anamnestic data may have been lacking

Obviously I took into account a number of symptoms and signs as well as those listed as cardinal criteria. A diagnosis of chronic pyelonephritis was not however made unless at least three of them were fulfilled irrespective of which other renal symptoms were present. This also applied to the cases in which all eight criteria could not be tested. In a few patients who did not fulfil even three criteria the diagnosis was nevertheless considered as highly probable on the grounds of other symptoms and signs. They are accounted for in a separate group (A2) denoted as acute or suspected chronic pyelonephritis.

In addition to the diagnostic criteria described in detail in the following and the patients' statements attention was also focused on the existence of e.g. concretions or malformations of the urinary tract, hypertrophy of the prostate, diabetes mellitus and other complicating diseases. These are listed in Table IV. For the classification of the material reference is made to Chapter V.

Naturally one cannot rule out with certainty the existence of chronic pyelonephritis in individual patients in the other groups of renal disease investigated as comparative material.

The reverse — i.e. the existence of another renal disease dominated by superimposed chronic pyelonephritis — can also apply. The coexistence of chronic pyelonephritis and renal tuberculosis was reported by e.g. Jenni (115). My series contains two such cases (nos 113, 114). They were not included in the chronic pyelonephritis group even though they fulfilled the criteria. In recent years chronic glomerulonephritis has become less common in Sweden (10b).

For this reason among others it is highly improbable that any patient with silent glomerulonephritis and superimposed pyelonephritis would have been included in the present series. In two cases (nos 108, 116) this possibility could not however be definitely ruled out and they were therefore assigned to the chronic pyelonephritis group.

The clinical features and results of examinations were divided into the following four main groups:

A Renal symptoms or results of renal function tests which at any rate when present concurrently strengthen the suspicions of chronic pyelonephritis — denoted here as *cardinal criteria*

B General symptoms of renal disease

C General signs of renal disease

D General signs of disease

A Cardinal Criteria

1. *Certain anamnestic data*

Among these data were *dysuria* and/or *pollakiuria* either at the time of examination or in the history. Anamnestic data on these symptoms were taken into account only if they had been confirmed by a physician and had been associated with pyuria and/or bacteriuria. The coexistence of *fever* and a feeling of *tension in the flank* was a strong reason to conclude that the above mentioned complaints were not merely signs of infection or irritation of the lower urinary tract (cystitis and/or urethritis) but that the renal tissue was in fact involved. An attack of *hyperpyrexia* without local symptoms and signs was on the other hand counted as a sign of acute pyelonephritis only if massive pyuria and bacteriuria were demonstrable concurrently. Brod (29) found in his

DIAGNOSIS OF CHRONIC PYELONEPHRITIS

It is often difficult to establish an unquestionable diagnosis of chronic pyelonephritis, and in almost every case there is in fact, some degree of uncertainty. In many cases in which the diagnosis is regarded as fairly incontrovertible, one or more of the clinical features or results of tests usually considered as significant are lacking. On the other hand none of the most characteristic features is pathognomonic of chronic pyelonephritis. Consequently, when a differential diagnosis is to be made between chronic pyelonephritis and other renal diseases one can seldom avoid some degree of subjectivity in the evaluation. In order to disclose as many anamnestic data as possible, and to permit uniform evaluation of the case material all patients with suspected chronic pyelonephritis and other renal diseases who were admitted to the Renal Clinic of our hospital had therefore, to reply to a detailed questionnaire.

On the basis of the data obtained in this way thorough physical examination and various functional tests I set up eight cardinal criteria of chronic pyelonephritis. According to other authors (10a and b, 28, 29, 30, 31, 37, 38, 135, 194) and personal experience in a large series of cases these features are particularly common in the disease in question. When at least three of these criteria were unquestionably fulfilled the patient was denoted as having chronic pyelonephritis. These eight cardinal criteria were:

- 1 A history of dysuria and/or pollakuria combined either with fever and pain in the flank, or with pyuria and/or bacteriuria
- 2 Tenderness to palpation over one or both loins
- 3 Decreased ability to acidify the urine
- 4 Reduced renal concentration ability
- 5 Characteristic interstitial changes at histologic examination of renal tissue
- 6 Bacteriuria
- 7 Characteristic appearance of the urinary sediment
- 8 Characteristic roentgenologic changes in the kidney and renal pelvis

It must be stressed once more that none of these symptoms or signs singly can be regarded as characteristic of chronic pyelonephritis but that the diagnosis is strongly supported if they are present concurrently. Thus dysuria alone combined with a feeling of tension in the flank does not suffice for a diagnosis. If however bacteriuria also exists the first mentioned feature carries much more weight. According to experienced workers, even the microscopic features of a biopsy specimen may occasionally be misleading (112). In 24 of my 80 cases of chronic and acute non obstructive pyelonephritis (groups A1, A2) the occurrence of all eight cardinal criteria could not be investigated. For example a renal biopsy may have been unsuccessful, it may have been impossible to determine the acidifying ability of the urine in cases with urea splitting bac

Placematia	Cloudy (10 smth. & un. #)	Pregnancy (no.)	Phenacetin abuse	Pres. vs bladder catheter.	BP (mm Hg) 1/2 hr. 25 (max) or on admission	BP (mm Hg) 24 hours (min)	Ocular fu di (Keith & Wagener) FII	Serum creatinine (mg/100 ml)	Water load ng test (mOsm/dl)	Proteinuria	Clearances ml min 1.73 m RSA			Chemical signs of disease	Intercurrent diseases
											PAH	Inulin	Endog creatinine		
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32

GROUP A1 Chronic non obstructive pyelonephritis (≥ 3 cardinal criteria)

0	0	III	0	0	130/85	105/75	I	18	140	(+)	(270)	(15)	(49)	+	Art. hypert.
+	+	III	0	+	2.0 140	160 100	III	18	195	(+)	111	24	49	+	Art. hypert.
0	0	I	0	0	170 110	130 80	0	16	95	(+)	136	-	69	+	
0	0	0	0	0	100 60	90 50	0	10	75	(+)	(145)	(31)	(4)	+	Art. hypert.
0	0	0	0	0	120 75	90 50	0	17	97	(+)	(49)	(52)	(51)	+	
0	0	0	0	0	160 110	130 90	0	28	118	+	(61)	(14)	(22)	+	
0	0	0	0	0	135 90	105 65	0	2.1	287	(+)	275	-	31	+	
0	+	III	0	0	110 65	-	0	2.2	170	(+)	(117)	(29)	(70)	+	Art. hypert.
0	0	0	0	0	150 100	130 85	III	0.9	85	0	375	-	130	+	
0	0	II	+	+	130 90	170 79	0	0.9	75	0	290	-	10	+	
0	0	0	0	0	185/100	155 85	0	1.6	107	0	(404)	(87)	(60)	+	Art. hypert.
0	+	0	(+)	0	230 115	155 90	II	2.3	204	(+)	(167)	(40)	(44)	+	
0	0	0	0	0	110 65	140 80	0	1.2	108	0	(146)	(12)	(5)	+	
0	0	0	0	0	145 80	105 65	I	2.1	100	0	(141)	(50)	(39)	+	Art. hypert.
0	0	0	0	0	140 110	105 85	0	0.8	89	0	-	-	-	+	
0	0	0	0	0	110 70	85 55	0	4.2	163	+	(73)	(17)	(23)	+	
0	0	II	(+)	0	150 80	-	0	1.1	84	0	(304)	(78)	(57)	+	
0	0	0	0	0	115 90	105 80	0	1.3	175	(+)	-	-	-	+	
0	0	0	0	0	140 80	-	I	1.4	90	0	(403)	(74)	(72)	+	
0	0	0	0	0	135 75	105 55	I	2.6	105	+	94	-	6	+	
0	0	0	0	0	145 90	110 70	0	1.8	90	0	(198)	(35)	(53)	+	
0	0	0	0	0	165 95	170 70	0	1.9	85	(+)	(291)	(55)	(56)	+	
0	+	III	+	0	170 110	145 90	0	2.0	113	(+)	706	(35)	39	+	Art. hypert.
0	0	0	0	0	180 80	105 65	0	1.8	85	(+)	-	(23)	(14)	+	B.L. renal papill. necr.
0	0	IV	+	0	160 110	140 80	-	1.9	117	(+)	171	-	29	+	
0	0	I	0	0	30 125	95 100	I	2.3	117	(+)	121	(27)	41	+	Art. hypert.
0	0	0	0	0	100 100	190 90	I	2.4	150	(+)	204	(27)	33	+	B.L. renal papill. necr.
0	0	(+)	+	0	125 140	165/130	II	1.9	-	(+)	165	(85)	83	+	Art. hypert.
0	0	0	0	0	170 100	170 75	I	1.8	305	-	67	-	64	+	B.L. renal papill. necr.
0	0	0	0	0	160 100	-	III	1.4	-	0	-	-	-	+	Art. hypert.
0	0	II	0	0	145 85	130 75	0	2.6	9	(+)	179	-	47	+	B.L. renal papill. necr.
0	0	0	0	0	155 90	130 75	0	2.2	75	+	-	-	-	+	
0	0	0	0	0	185 75	100 60	0	1.0	75	0	(616)	(115)	(93)	0	
0	0	III	+	+	155 75	105 60	0	2.0	75	(+)	118	18	35	+	B.L. renal papill. necr.
0	0	0	0	0	145 80	-	0	1.7	75	(+)	330	-	105	+	
0	0	III	+	+	140 85	170 70	0	1.2	60	0	31	-	8	+	
0	0	0	0	0	150 85	105 75	0	1.3	90	0	371	(56)	48	+	
0	0	0	0	0	155 85	85 45	0	1.8	100	(+)	186	(83)	6	+	
0	0	0	0	0	95 45	-	0	1.1	25	(+)	(51)	83	74	+	
0	0	0	0	0	170 110	170 95	0	1.8	207	0	46	-	116	+	Art. hypert.
0	0	0	0	0	10 130	190 170	0	2.7	145	+	(98)	(6)	(40)	+	
0	0	0	0	0	160 90	-	III	1.3	60	0	441	-	97	+	
0	0	0	0	0	195 95	-	II	1.8	-	(+)	14	-	33	+	
0	0	0	0	0	180 105	145 60	II	10.5	10.5	0	(19)	(40)	(50)	+	Art. hypert.
0	0	0	0	0	160 105	104 70	0	1.6	4	(+)	(601)	(1.8)	(65)	+	Diab mell.
0	0	0	0	0	170 95	105 75	-	1.7	170	+	(374)	(49)	(105)	+	Art. hypert.
0	0	0	0	0	130 85	105 70	0	1.3	75	0	34	-	115	+	Art. hypert.
0	0	0	0	0	180 110	145 95	0	1.3	60	(+)	-	-	-	+	B.L. renal papill. necr.
0	0	0	0	0	190 95	130 90	I	0.8	94	+	14	-	-	+	

Table IVa Cardinal criteria and salient data
Symbols as in Table IVb

Case no	Case Record no	Age at invest (yrs)	Sex	Cardinal criteria of pyelonephritis										Histologic exam B = Biopsy R = Resection or ectomy A = Autopsy	Bacteriuria no/ml	Urinary sediment	Roentgen exam
				Cystitis without fever	Cystitis with fever	Chills without cystitis	Hist. of lo n pain	Local tenderness to palpation	Ac dif abil (pft)	Max conc ab litv (mOsmol/lit)							
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15			
GROUP A1 Chronic non obstructive pyelonephritis (≥ 3 cardinal criteria)																	
1	2261/60	41	F	0	+	0	+	0	536	535	R +	700 000 Ec	+	+	+	+	
2a	9952/58	38	F	+	+	+	+	0	498	383	B 0	26 mill Ec	+	+	+	+	
3	2593/62	40	F	+	+	+	+	0	534	465	B 0	400 000 Ec	+	+	+	+	
4a	7653/65	45	F	+	+	+	+	0	34	313	B +	10 m ll Ec	+	+	+	+	
4b		47	F	+	+	+	+	0	568	520	B +	2 mill Ec	+	+	+	+	
5	14449/60	31	F	+	0	0	0	0	52	369	A +	51 mill Ec	+	+	+	+	
6a	7106/61	43	F	0	0	+	+	+	51	—	B 0	16 mill Ec	+	+	+	+	
6c		46	F	+	+	+	+	+	575	500	B +	24 m ll Ec	+	+	+	+	
7a	4710/61	66	F	+	+	0	(+)	0	513	390	B +	0	+	+	+	+	
7b		68	F	+	+	0	0	0	515	577	B +	10 5 mill Ec	+	+	+	+	
8a	1415/61	49	F	+	0	0	0	0	496	412	B +	0	+	+	+	+	
8b		52	F	+	+	+	+	+	515	354	B +	>100 000 Ec	+	+	+	+	
9a	4413/61	57	F	+	+	+	+	0	525	134	B +	0	+	+	+	+	
9b		60	F	+	0	0	0	0	53	747	B +	96 5 mill Ec	+	+	+	+	
10	9141/58	22	F	+	0	0	0	0	53	—	B +	0	+	+	+	+	
11	7392/58	38	F	—	—	(+)	+	+	40	317	B 0	19 mill Ec	+	+	+	+	
12	9317/58	55	F	0	+	+	0	0	40	254	B 0	>100 000 Ec	+	+	+	+	
13a	7467/61	55	F	0	+	+	0	0	305	240	B +	75 m ll Ec	+	+	+	+	
13b		58	F	0	0	+	(+)	0	302	620	B +	0	+	+	+	+	
14a	540/61	26	F	0	0	+	(+)	0	335	505	B +	68 mill Aa	+	+	+	+	
14b		28	F	+	+	0	+	+	326	573	B +	0	+	+	+	+	
15	5243/61	51	F	+	+	0	+	+	546	422	B +	10 m ll Ec	+	+	+	+	
16	4655/61	62	F	+	+	0	+	0	520	430	B +	120 m ll Ec	+	+	+	+	
17	9915/59	35	F	+	+	0	+	0	436	415	B +	75 mill Ec	+	+	+	+	
18	239/60	65	F	+	+	0	+	+	493	395	B 0	90 m ll Ec	+	+	+	+	
19a	16401/63	55	F	+	+	0	+	0	39	409	B +	15 mill Ec	+	+	+	+	
19b		58	F	+	+	0	+	0	449	393	A +	0	+	+	+	+	
20a	8471/62	40	M	+	+	—	+	0	—	480	—	>100 000 Aa	+	+	+	+	
20b		53	M	+	+	—	+	0	—	—	—	34 m ll Aa	+	+	+	+	
21	4391/67	45	F	0	0	0	0	+	54	352	B +	60 mill Ec	+	+	+	+	
22a	8536/61	30	F	0	+	0	+	0	55	365	B +	>100 000 Ec	+	+	+	+	
22b		33	F	0	+	0	+	0	55	365	B +	20 mill Ec	+	+	+	+	
23	8198/61	41	F	+	0	0	0	0	551	940	B +	73 mill Ec	+	+	+	+	
24	1717/59	45	F	+	+	0	+	+	515	340	B +	7 m ll Ec	+	+	+	+	
25a	1086/62	44	F	+	0	+	+	0	550	338	B +	>100 000 Ec	+	+	+	+	
25b		46	F	+	0	+	+	0	812	450	B +	18 m ll Pm	+	+	+	+	
26a	6423/63	45	F	+	+	+	+	0	565	390	B 0	100 000 Ec	+	+	+	+	
26b		46	F	+	+	+	+	0	633	405	B 0	100 000 Ec	+	+	+	+	
27	9600/59	31	F	+	+	0	+	0	505	865	B 0	10 m ll Ec	+	+	+	+	
28a	16940/62	31	F	+	+	0	+	0	56	—	B +	>100 000 Ec	+	+	+	+	
28b		31	F	+	+	0	+	0	474	395	A +	0	+	+	+	+	
28c		32	F	+	+	+	+	+	56	415	B (-)	0	+	+	+	+	
28d		32	F	+	+	+	+	+	56	415	B (-)	25 m ll Pm	+	+	+	+	
29a	445/67	54	F	+	+	+	+	+	50	513	B 0	>100 000 S alb	+	+	+	+	
29b		55	F	0	0	+	+	+	445	450	B 0	0	+	+	+	+	
29c		30	F	0	0	+	+	+	520	50	B 0	0	+	+	+	+	
30a	1868/61	34	F	0	0	+	+	+	505	765	B 0	>100 000 Aa	+	+	+	+	
30b		34	F	0	0	+	+	+	545	475	B +	1 m ll Ec	+	+	+	+	
31	7739/61	48	M	0	+	0	+	+	505	765	B 0	>100 000 Aa	+	+	+	+	
32	6606/62	40	M	0	+	0	+	+	545	475	B +	1 m ll Ec	+	+	+	+	
33	1835/62	54	F	0	+	0	(+)	+	465	761	B 0	>100 000 Ec	+	+	+	+	
34	5846/62	66	F	0	+	0	(+)	+	689	565	B +	190 m ll Pm	+	+	+	+	
35	14165/62	43	F	0	0	0	0	0	520	355	B A +	0	+	+	+	+	

Case no	1	17	18	19	20	21	22	23	24	25	26	27	Clearances ml min 1.73 m ² BSA			Central sites of disease
													PAH	Insulin	Endog creatinine	
		Stemur a	Cloudy at end of urine	Pregnancies (n)	Phenacetin abuse	Previous bladder catheter	BP (mm Hg) 24 hours (ma) of on admission	BP (mm Hg) 4 h (mm)	Ocular fundi (Ka & Wagener) FH	Serum creatinine (mg/100 ml)	Water load mg test (mOsmol/l)	Proteinuria				

GROUP A1 Chronic non-obstructive pyelonephritis (≥ 3 cardinal criteria)

1	+	+	III	0	+	130 85	105 75	I	1.6	140	(+)	(770)	(53)	(49)	+
2	+	0	II	0		220 140	160 100	II	1.8	195	++	211	5	49	+
3	+	0	III	0		170 110	130 80	II	1.6	94	(+)	136	5	49	+
4a	0	0	I	0		100 80	90 60	0	2.0	97	(+)	(148)	(31)	(4)	+
4b	0	0		0		120 75	90 60	0	1.7	75	(+)	(249)	(5)	(31)	+
5	+	0	I	0	0	160 110	130 90	0	2.8	118	+	(61)	(14)	(3)	+
6a	0	0	0	0	0	125 90	105 65	0	2.1	187	(+)	225	—	3	+
6b	0	0	0	0	0	110 65	105 65	0	2.2	170	(+)	(117)	(79)	(70)	+
7a	0	—	III	0	0	150 100	130 85	III	0.9	83	—	373	—	130	+
7b	0	—	III	0	0	150 90	125 90	0	0.9	73	0	290	—	80	+
8	0	II	II	+	+	185 100	155 85	0	1.6	107	0	(404)	(87)	(60)	+
8b	0	0	0	+	+	230 115	155 90	II	3	204	(+)	(167)	(40)	(44)	+
9a	+	0	0	(+)	0	110 65	140 80	0	1.2	108	0	(46)	(17)	(5)	+
9b	0	0	0	0	0	145 80	140 80	I	2.1	100	0	(141)	(30)	(39)	+
10	0	0	0	0	0	140 110	105 85	0	0.8	88	0	—	—	—	+
11			I	—	0	110 70	85 55	0	4.2	163	+	(73)	(17)	(73)	+
12			0	0	0	150 80	—	—	1.1	84	0	(304)	(78)	(57)	+
13a	0	0	II	(+)	0	115 90	105 80	0	1.3	173	(+)	—	—	—	+
13b	0	0	0	0	0	140 80	—	I	1.4	90	0	(403)	(74)	(72)	+
14b	0	0	0	0	0	155 73	105 55	I	2.6	105	+	94	—	6	+
14c	0	0	0	0	0	145 90	110 70	0	1.8	90	0	(198)	(35)	(53)	+
15	0	0	I	0	0	165 95	120 70	0	1.9	85	(+)	(291)	(55)	(56)	+
16			III	0	0	170 110	145 90	0	2.0	113	(+)	—	(35)	39	+
17			0	+	0	105 80	105 65	0	1.8	81	(+)	—	(3)	(34)	+
18			IV	—	0	160 110	140 80	0	1.9	117	(+)	171	—	29	+
19a	0	0	I	—	0	30 1.5	195 100	I	2.3	117	(+)	121	(27)	41	+
19b	0	0	0	—	0	230 100	190 90	II	2.6	150	(+)	104	(27)	32	+
20a			(+)			225 140	165 130	II	1.9	—	(+)	145	(15)	63	—
20b						150 100	170 75	I	1.8	305	—	227	—	64	—
21			II	0	0	160 100	—	III	1.4	—	0	—	—	—	+
22			II	0	0	145 88	125 75	0	6	9	(+)	179	—	47	+
23			II	0	0	155 90	130 75	—	—	75	+	—	—	—	+
24	0	0	II	0	+	115 75	100 60	0	1.6	73	0	(616)	(115)	(93)	0
25	0	0	III	+	+	155 75	105 60	0	2.0	65	(+)	118	38	35	+
26	0	0	III	+	+	145 80	—	0	1.5	75	(+)	330	—	103	+
27	0	0	0	0	0	140 85	120 70	0	1	60	0	321	—	8	—
28	0	0	0	0	0	100 85	105 75	0	1.3	90	0	3	(56)	48	+
29	0	0	0	0	0	135 80	85 45	0	1.8	100	(+)	186	(83)	6	+
30	0	0	II	0	0	95 65	—	0	1.1	25	(+)	(312)	83	76	+
31	0	0	0	0	0	170 110	140 95	0	1.8	207	0	46	—	116	+
32	0	0	0	0	0	10 130	190 170	0	1.7	145	+	(98)	(6)	(40)	+
33	0	0	0	0	0	160 90	—	0	1.3	60	0	441	—	97	+
34	0	0	0	0	0	105 95	—	0	1.8	—	(+)	14	—	33	+
35	0	0	0	0	0	180 105	145 60	0	1.5	105	0	(19)	(40)	(50)	+
36	0	0	0	0	0	160 105	105 70	0	1.6	4	(+)	(601)	(1.8)	(65)	+
37	0	0	0	0	0	140 95	105 75	0	1.7	170	++	(574)	(19)	(80)	+

Table IVa (continued)

Case no	Case Record no	Age at invest (yrs)	Sex	Cardinal criteria of pyelonephritis										Bacteriuria no/ml	Urinary sediment	Reorganism exam	No pos critr a/No critria tested
				Cystitis without fever	Cystitis with fever	Chills without cystitis	Hist of loin pain	Loin tenderness to palpation	Acidur abnl (pH)	Max conc ability (mOsmol/lit)	Histologic exam B = Biopsy R = Resection of ectomy A = Autopsy						
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
GROUP A1 (continued)																	
36	1303/61	49	F	+	+	0	+	0	507	802	B 0	>100 000 Ec.	+	0	38		
37	8837/61	35	F	+	+	0	+	0	545	680	B 0	4 mill Ec	+	0	48		
38	4927/61	39	M	+	+	0	+	+	510	514	B +	3 mill Ec	+	0	78		
39	† 403/64	31	F	+	0	0	0	0	510	385	A +	0	0	0	38		
40	3230/62	51	F	+	+	0	+	+	—	—	B +	>100 000 Ec.	+	+	66		
41	9412/60	45	F	0	(+)	+	0	0	534	570	B +	900 000 Ec	+	+	78		
42	7672/62	54	F	0	+	0	+	+	475	675	B +	>100 000 Ec.	+	+	58		
43	2487/61	58	F	0	+	0	+	+	475	416	B +	41 mill Ec	+	+	68		
44	1604/63	27	F	+	0	0	(+)	0	568	345	B +	0	0	+	48		
45	3010/61	48	F	+	0	0	0	0	—	902	B +	0	+	+	47		
46	45/60	37	F	0	(+)	+	+	+	53	345	—	8 mill Ec.	+	+	77		
47	1453/60	60	F	0	+	+	+	0	522	475	B 0	15 mill Ec.	+	0	47		
48	8666/62	18	F	+	+	0	+	0	491	1100	B +	>100 000 Ec.	+	+	38		
49	6974/62	24	F	+	+	0	+	+	—	950	B +	>100 000 Ec.	+	+	56		
50	6972/62	49	M	0	+	0	+	0	—	—	B +	3.4 mill Ec.	+	+	47		
51	6474/62	42	F	0	+	—	+	+	—	430	—	12 mill Ec.	+	+	66		
52	2227/63	34	F	+	0	0	+	0	600	600	B +	0	0	+	47		
53	6879/62	56	F	+	0	0	+	+	547	840	B +	13* 000 Pm	+	+	57		
54	2369/61	41	F	+	0	—	+	0	575	410	B +	4 mill Ec	+	+	78		
55	2409/62	24	F	0	+	—	+	+	525	945	B 0	>100 000 Ent.	0	+	58		
56	2962/62	46	F	0	+	0	+	+	—	395	B +	40 mill Ec.	+	+	77		
57	4681/61	54	F	0	+	0	0	0	510	725	B 0	>100 000 P.	+	0	48		
58	2960/62	46	F	0	+	0	+	0	475	675	—	2 mill Ec	+	+	47		
59	6632/62	42	F	+	+	0	+	+	651	345	—	>100 000 Ec.	+	+	77		
60	6412/62	34	F	+	+	0	+	+	512	458	B +	1 mill Ec.	0	+	68		
61	3550/59	53	F	+	+	0	+	0	53	370	B +	>100 000 Ec.	+	+	78		
62	2406/59	47	F	+	+	0	+	+	53	535	B (+)	60 mill Ec.	+	0	78		
63	4070/64	35	F	0	0	0	+	0	505	545	—	600 000 Ec.	+	+	57		
64	5416/59	35	F	0	0	0	+	0	54	535	B 0	60 mill Ec.	+	+	47		
65	7872/61	34	F	0	+	+	+	+	50	492	B 0	>100 000 Ec	+	+	68		
66	4358/59	52	F	+	0	0	0	0	53	470	B +	800 000 Ec.	+	+	73		
67	2598/62	41	F	+	+	0	+	+	465	670	B +	>100 000 Ec.	+	+	68		
68	1599/64	67	F	+	0	0	0	0	500	835	B +	110 mill P.	+	0	48		
69	359/65	44	F	+	0	0	0	0	656	390	B +	0	+	+	48		
GROUP A2 Acute and suspected chronic non obstructive pyelonephritis (< 3 cardinal criteria)																	
70	901/60	35	F	+	0	0	+	0	532	1030	B 0	0	0	0	18		
71	9234/58	33	F	(+)	0	0	+	0	50	814	B 0	0	0	0	18		
72	8286/60	20	F	0	0	0	0	0	532	1165	B 0	75 mill Ec.	0	0	28		
73	7983/60	45	F	+	+	0	(+)	0	480	905	—	3 mill Ec.	0	0	17		
74	1153/60	62	F	+	0	0	0	0	—	980	B 0	0	0	0	16		
75	68/62	39	M	0	0	0	0	0	—	868	B 0	>100 000 Ec.	+	0	17		
76	9232/61	63	F	+	+	0	+	0	467	870	B 0	>100 000 Ec.	0	0	28		
77	6947/60	20	F	0	0	0	—	0	519	1120	B +	0	0	0	18		
78	5339/64	43	M	0	0	0	0	0	—	415	B +	0	0	0	27		
79	8697/61	41	F	+	0	0	0	0	52	1090	B 0	0	0	0	18		
80	4734/61	26	F	+	+	0	+	0	488	1170	B 0	0	+	0	18		

Cloudy ill smell ng ur	Pregnancies (no.)	Phenacetin abuse	Previous bladder milt	BP (mm Hg)/24 hours or on admission	BP (mm Hg)/24 hours	Ocular fundi (Keith & Wagener) 1	Serum creatin in (mg)	Water load ng test (mO mol l)	Proteinur a	MAH	Inulin	Endog creatinine	General signs of di	Intercu disea
18	19	20	21	22	23	24	25	6	27	28	29	30	31	3

GROUP A1 (continued)

+	III	0	-	145/90	100/65	0	14	67	0	383	-	93	+	
+	IV	0	0	135/80	115/65	0	11	50	0	470	-	116	+	
				130/90	100/60	II-III	2.0	157	(+)	370	-	56	+	BL re pap II
+	II	III	+	170/100	120/85	0	4.0	140	+	89	-	50	+	BL re pap II
				100/65	80/50	0	1.5	-	0	-63	-		-	
+	V	+	-	165/95	170/75	I	0.9	340	(+)		-	-	+	
+	II	+	III	140/95	120/60	0	1.3	265	0	283	-	61	+	Art b
0	0	0	0	180/120	135/95	0	1.5	88	(+)	102	-	30	+	Pap II
													-	Art b
	I	+	-	163/105	125/85	0	2.3	310	+	215	-	47	+	Pap II
+	0	0	0	255/150	170/100	35	5.0	70	0	450	-	98	+	Pap II
													-	Art I
0	0	0	0	140/85	120/70	0	2.0	105	+	207	-	45	+	
0	0	0	0	180/100	135/70	I	1.3	270	(+)	418	-	113	+	Art I
0	0	0	0	135/85	100/70	0	1.1	45	0	-	-	-	+	
0	0	0	0	115/70	95/50	0	0.9	75	0	539	-	1.5	+	
+				130/85	115/70	0	1.9	310	+		-	19	+	
+	0	0	0	150/80	115/65	I	2.5	134	0		-	-	+	
0	0	0	0	190/105	130/80	0	1.4	115	0	295	-	49	+	Art I
0	0	0	0	115/85	110/70	0	1.4	78	0	485	-	93	+	
+	1	+	+	160/85	130/70	0	1.6	100	(+)	40	-	63	+	
+				107/70	95/40	0	1.0	1.5	0		-	-	+	
+	0			120/80	95/55	0	1.7	94	(+)	184	-	51	-	BL pap I
0	0	0	0	135/85	95/60	0	1.1	67	0	295	-	75	-	
0	0	0	0	120/90	100/65	0	1.3	57	0	3.4	-	8	+	BL pap I
0	0	0	0	115/75	105/65	I	6.8	200	+	37	-	9	+	
0	0	0	0	1.5/85	105/60	0	2.6	87	0	173	-	43	-	BL pap I
0	()	0	+	160/90	1.0/70	I	1.8	20	(-)	155	-	35	+	BL pap I
0	0	0	0	130/80	95/40	0	1.7	110	0	503	-	101	+	
0	0	0	0	135/80	115/70	0	2.4	110	0	81	-	81	+	
0	0	0	0	170/88	110/70	0	1.4	100	(+)	799	-	84	+	
0	+	0	0	125/90	115/70	0	1.7	155	(+)	133	-	71	+	
0	0	0	0	170/75	-	-	1.6	85	(+)	-	-	-	+	
0	0	0	0	155/100	125/80	I	2.4	80	0	30	-	59	+	Art
0	0	0	0	130/88	170/65	0	0.8	85	(+)	-	-	-	+	
0	0	0	0	115/45	1.5/85	0	4.6	240	-	-	-	-	+	

GROUP A2 Acute and suspected chronic non-obstructive pyelonephritis (< 3 cardinal criteria)

0	()	0	0	115/65	85/55	0	0.8	145	0	(717)	(163)	(87)	+	
0	0	0	0	115/70	105/55	0	1.4	50	0	-	-	-	+	
0	0	0	0	120/65	95/50	0	0.9	60	(+)	-	-	-	+	
0	0	0	0	125/90	110/65	0	1.0	60	(+)	564	-	19.1	+	
0	0	0	0	120/80	110/70	0	0.7	80	0	635	-	153	+	
0	0	0	0			0	0.9	-	(+)	3.1	-	103	+	
+	0	0	0	160/95	130/70	0	1.1	100	0	385	-	60	+	
0	0	0	0	130/85	90/65	0	1.1	73	0	386	-	90	-	
0	0	0	0	10/90	175/80	I	4.4	1.8	+	-	-	-	-	

Table IVb Cardinal criteria and salient data

Case no	Case Record no	Age at invest (yrs)	Sex	Cardinal criteria of pyelonephritis										Urinary sediment	Rectal exam	No. pos. cultures/No. criteria tested
				Cystitis without fever	Cystitis with fever	Chills without cystitis	Hist of loin pain	Loin tenderness to palpation	Acidif abil (pH)	Max conc Abnity (mOsmol/kg)	Histologic exam B = Biopsy R = Resection of ectomy A = Autopsy	Bacteriuria no./ml				
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
GROUP B1: Chronic pyelonephritis with signs of urinary tract obstruction (≥ 3 cardinal criteria)																
81	4978/58	51	F	+	0	0	0	0	5.6	445	B +	125 mill Ec	+	+	x	7.8
82a	3824/63	47	F	+	+	-	+	+	7.1	331	B +	16.5 mill Ec.	+	+	x	8.8
82b		48										900 000 Pm				
82c		49							6.00	385	-	> 100 000 Pm	+	+	x	
82d		51							6.38	463	-	900 000 Pm	+	+	x	
83a	↑ 7681/59	42	F	+	+	0	+	+	6.0	345	-	> 100 000 Ec Aa	+	+	x	8.8
83b		42							6.0	345		66 mill Ec Aa	+	+	x	
83c		43							6.23	310	A +	167 mill Aa	+	+	x	
84a	7775/62	29	F	0	0	+	+	0	3.6	277	-	50 mill Ec	+	+	x	6.7
84b		32							5.05	360	-	17 mill Ec	+	+	x	
85	8804/60	60	M	+	+	0	(+)	0	4.85	670	B +	> 100 000 Ec.	+	+	x	6.8
86a	1791/62	54	F	+	+	0	+	+	5.15	425	B +	> 100 000 Pm	+	+	x	8.8
86b		56										140 mill Ec	+	+	x	
86c		57							6.04	495	-	2.6 mill Pm	+	+	x	
87	5477/61	53	F	0	(+)	+	+	0		244	B +	> 100 000 Ec	+	+	x	6.7
88	3883/62	53	F	0	(+)	+	+	+	4.92	940	-	2.5 mill Ec	+	+	x	5.7
89	1293/59	52	F	+	+	0	0	0	6.6	505	R +	100 000 Pm	+	+	x	7.8
4c	7655/65	48	F	+	+	+	+	0	3.84	513	B +	700 000 Ec Pm	+	+	x	7.8
90a	↑ 241/65	58	F	+	+	+	+	+	6.38	-	B +	> 100 000 Pm	+	+	x	7.7
90b		61									A +	> 100 000 Pm	+	+	x	
91	468/61	55	F	0	+	0	+	0	-	590	R +	0	0	+	x	4.7
92	1870/63	43	F	0	+	0	+	0	-	575	B R +	3 mill Ec	+	+	x	6.7
93	1627/59	61	F	0	(+)	+	+	+	5.5	685	-	1 mill Ec.	+	+	x	6.7
94	8402/61	42	F	+	(+)	0	+	+	5.95	385	B -	7 mill Ec.	+	+	x	8.8
95	4234/62	51	F	+	0	0	0	0	5.77	477	-	0	+	+	x	4.7
96	2600/61	22	F	0	+	0	+	+	5.15	1070	-	> 100 000 Ec	+	0	x	4.7
97	1104/61	37	F	0	0	0	0	0	6.2	575	R +	> 100 000 Ec	+	+	x	6.8
98	2208/61	44	F	+	+	0	(+)	0	5.40	980	B +	> 100 000 Ec	+	+	x	6.8
99a	6357/63	45	F	0	0	+	0	+	5.0	540	B +	307 000 Pm	+	+	x	7.8
100	4107/60	16	F	0	+	0	+	+	5.40	1095	-	170 000 S aur	+	+	x	4.7
101	3439/60	28	F	+	+	0	+	+	5.06	715	-	> 100 000 Ec				6.7
102a	2648/61	52	F	0	+	0	(+)	+	5.50	485	-	230 000 Aa	0			4.7
103	9118/61	28	F	+	+	0	+	+	5.00	567	B -	> 100 000 Ec	0			6.8
104	1607/62	37	F	0	0	+	(+)	0	5.69	480	B -	> 100 000 Ec	+			6.8
105a	3636/60	38	F	0	0	+	(+)	0	5.35	459	-	270 000 Pm				6.7

Symbols
 0 Nothing Pathologic observed or answer to question negative
 + Pathologic finding or answer to question positive
 (+) Doubtful positive finding or doubtful positive answer
 x Pathologic finding without relevance to pyelonephritis
 - Not investigated or questioned

Column 1
 a, b, c and d denote examinations on different occasions in the same patient

Column 13
 Ec *Escherichia coli* coliform organisms
 Aa *Aerobacter a. raeorum*
 Pm *Proteus mirabilis* P. vulgaris
 S aur *Staphylococcus aureus*
 S alb *Staphylococcus albus*
 Ent *Enterococci*

Patient's History										Clearances and in 173 ml BSA										Intercurrent diseases	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Cloudy in morning urine	Pregnancy (no.)	Phenacetin abuse	Previous bladder catheteriz.	BP (mm Hg) 4 hours (max) or on admission	BP (mm Hg) 4 hours (min)	Quaker fundi (Keith & Wagener) F15	Serum creatinine (mg/100 ml)	Water load test (mOsm 1 hr)	Proteinuria	PAH	Inulin	Endog creatinine	General signs of disease								
+	+	0	0	130/75	110/60	-	1.8	228	(+)	(33)	(48)	(65)	+	Hydronephrosis R. Vaso-ureter reflux R. B1 nephrolith							
0	(+)	1	0	150/85 130/70	100/60 110/60	0	2.0 2.4	129	(+) (+)	(119) 169	(18) 48	(15) 48	+								
-	-	0	0	160/90 150/85	115/70 110/70	0	2.3 1.8	215 151	(+) (+)	(210) 184	(15) 47	(48) 27	+	B1 nephrolith.							
0	+	1	0	140/100 20/140	150/80 160/110	0	3.1 3.1	266 205	(+) ++	(58) (104)	(21) (20)	(31) (17)	+	Art. hypert. B1 renal papill. necr B1 nephrolith. Hypertroph. prost.							
0	0	0	+	165/90	135/80	1	1.7	135	+	12.	-	52	+	B1 renal papill. necr Nephrolith. L.							
0	-	1	+	140/85 160/85 175/90	125/70 125/65 130/65	1	1.9	145	(+) (+)	161 (119)	36 (74)	(17)	+								
0	+	0	+	100/70 265/160	85/40 200/105	0	1.2 1.3	80 120	(+) (+)	301 (4.5)	85 (73)	(7)	+	Nephrolith. L. Nephrolith. L. Art. hypert. Nephrolith. R. Nephrolith. L.							
0	0	1	0	135/80	-	0	1.6	84	(+)	(68)	(40)	(46)	0								
-	-	1	+	115/65 100/105	100/40 150/85	1	4.3 3.1	180	(+) ++	30 (78)	(12)	40 (44)	+	Art. hypert. B1 nephrolith.							
0	-	0	0	240/125 165/95	180/95 145/80	1	1.3 1.0	185 60	0 (+)	53 (109)	-	58 (81)	+	Art. hypert. Se. post perinephr. L. B1 renal papill. necr							
0	0	0	+	155/75 175/115	110/55 140/70	0	3.1 4.1	145 155	0 (+)	105 219	(19)	22 87	+	Nephrolith. L. Nephrolith. L. Art. hypert. B1 renal papill. necr							
0	0	0	-	140/100	110/60	0	1.1	170	0	56	-	58	+	Art. hypert. B1 renal papill. necr							
0	0	0	0	175/85	95/60	-	0.9	63	0	-	-	-	+	Leithersol th. R. Atony vesic. urina. Vaso-ureter reflux R.							
0	0	0	+	180/170	140/100	1	1.3	-	(+)	297	-	101	0	Art. hypert.							
0	0	0	0	130/80 140/95	95/60 115/75	0	1.3 1.9	61 90	(+) 0	536 171	-	93 44	0	Nephrolith. R. Nephrolith. R.							
0	0	0	-	135/70	100/60	0	1.1	31	-	349	-	15	+	Vaso-ureter reflux R.							
0	0	0	0	150/75 145/80	105/60 170/70	0	2.0 2.1	133 150	0 0	78 333	-	71 63	+	B1 nephrolith. Vaso-ureter reflux R. Art. hypert. Nephrolith. L. Hypertroph. R. Nephrolith. L. Hydronephrosis L. Renal papill. necr R. Nephrolith. R.							

(ol m 2 and)
When only one eye is used for the blood pressure at
denot th t on adon wion. When a dia volu pressure of
≥ 100 mm Hg is recorded on any occa on it is noted
n the table arterial hypert uion irrespective of any
f nd h acc.

Col 100 27

± ++ +++ denote slight, moderate and heavy pro-

€ / mgy 8-30

Bracketed values denote that the test was made directly after systemic catheterization.

Table IVb (continued)

Case no	Case Record no	Age at invest (yrs)	Sex	Cardinal criteria of pyelonephritis										Bacteriuria no/ml	Urinary sediment	Roentgen exam	No. of criteria/No. criteria tested
				Cystitis without fever	Cystitis with fever	Chills without cystitis	History of loin pain	Loin tenderness on palpation	Acid fast (git)	Max conc ability (mOsmol/kg)	Histologic exam B = Biopsy R = Resection or ectomy A = Autopsy						
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
GROUP B2 Renal disease (except pyelonephritis) with unilateral or mainly asymmetric involvement																	
106	4039/61	34	F	0	0	0	+	0	-	975	-	0	0	0	0	16	
107	3799/65	18	F	0	0	0	0	0	50	-	R +	0	0	0	0	17	
108	8562/59	36	F	0	0	0	+	+	491	535	R +	160 mill Ec	0	0	0	48	
109	8695/60	52	M	0	0	-	-	0	-	-	-	0	0	0	0	0	
110	6381/61	27	M	-	-	-	-	0	-	-	-	50 000 Ec	+	0	0	14	
111	9950/58	62	M	0	0	0	0	0	505	310	R 0	0	0	0	+	8	
112	6149/59	61	M	0	0	0	0	0	-	-	R +	0	0	0	0	16	
113	7373/60	61	F	0	0	0	0	0	604	737	R +	0 mill Ec	+	+	+	68	
114	1738/63	55	F	+	0	0	0	+	623	930	-	600 000 S alb	+	+	+	7	
115	5572/62	36	F	0	0	0	+	+	614	300	B 0 x	20 mill Aa	+	0	0	61	
116a	3227/61	17	F	0	0	0	0	+	-	-	B 0 x	0	0	0	0	18	
116b		18	F	0	+	0	0	+	484	1060	B +	0	0	0	0	0	
GROUP B3 Arterial hypertension with stenosis or aneurysm of the main renal artery																	
117a	3532/62	55	F	+	0	0	0	(+)	-	-	-	0	0	0	0	15	
117b	3532/62	56	F	+	0	0	0	0	-	-	-	0	0	0	0	0	
118	3794/58	30	F	0	0	0	0	0	-	-	R 0 x	0	0	0	0	0	
119	1069/62	66	M	0	0	0	0	0	-	-	-	0	0	0	0	0	
120	7084/61	53	F	0	0	0	-	0	-	1074	B 0 x	0	+	0	0	17	
121	2765/60	52	M	0	0	0	0	0	-	1025	-	0	0	0	0	0	
122	8361/61	36	F	+	0	0	(+)	0	-	-	A 0	0	0	0	0	0	
123	6008/62	61	F	0	0	0	0	0	-	-	-	0	0	0	0	0	
124	1282/62	53	M	0	0	0	-	0	-	-	-	0	0	0	0	0	
125	671/62	18	M	0	0	0	0	0	-	-	-	0	0	0	0	0	
126	431/62	63	F	0	0	0	0	0	-	-	-	0	0	0	0	0	
127	9186/61	64	F	0	0	0	0	0	-	-	-	0	0	0	0	0	
128	5803/62	45	M	0	0	0	0	0	-	-	-	0	0	0	0	0	
129	6358/62	54	F	+	0	0	0	0	-	-	-	0	0	0	0	0	
130	501/63	49	F	0	0	0	0	0	-	-	-	0	0	0	0	0	
131	3395/61	57	F	(+)	0	0	0	0	-	-	-	0	0	0	0	0	
GROUP B4 Renal disease with expected homogeneous involvement of both kidneys																	
132	2766/62	53	M	0	0	0	0	0	-	-	-	0	0	0	0	0	
133	5865/62	31	F	+	0	0	0	0	-	-	-	0	0	0	0	0	
134	1524/61	33	M	0	0	0	0	0	-	407	A 0 x	0	0	0	0	0	
135	2603/61	46	F	(+)	0	0	0	0	-	695	B 0 x	~100 000 P	0	0	0	7	
136	4667/58	45	M	0	0	0	(+)	0	52	584	B 0 x	0	0	0	0	18	
137	7143/58	23	M	0	0	0	0	0	-	745	A 0 x	0	0	0	0	15	
138 a	1198/60	35	F	0	0	0	+	0	-	565	B 0 x	0	0	0	0	16	
138 b		35	F	0	0	0	0	0	-	-	A 0 x	0	0	0	0	1	
139	9432/59	35	F	+	+	0	0	0	54	-	B 0	0	0	0	0	0	
140	2715/62	19	F	0	0	0	0	0	-	-	B 0 x	0	0	0	0	0	
141	2427/65	42	F	0	0	0	0	0	-	-	B 0 x	0	0	0	0	0	
142	7323/60	44	M	(+)	0	0	0	0	495	688	B 0 x	0	0	0	0	0	
143	4454/62	28	M	0	0	0	0	0	-	-	B A 0 x	0	0	0	0	0	
144	2707/60	19	F	0	0	0	+	+	520	835	B 0 x	74 mill Aa	0	0	0	16	
145	5058/64	34	F	0	0	0	0	0	-	318	B 0 x	17 mill Ec	0	0	0	7	
146	6307/65	31	M	0	0	0	0	0	54	560	B 0 x	0	0	0	0	18	
147	8963/60	35	F	0	0	0	0	0	-	-	-	0	0	0	0	0	
GROUP B5 Patients with symptoms from the lower urinary tract in whom involvement of the kidneys could not be ruled out																	
148	8890/58	37	F	0	0	0	0	0	-	675	B 0	0	0	0	0	15	
149	310/59	42	F	+	0	0	0	0	49	85	B (-)	0	0	0	0	17	
150	8825/60	41	F	+	0	0	0	0	-	1130	B 0	0	0	0	0	17	
151	8971/60	20	F	+	0	0	0	0	510	1000	B 0	500 mill Ec	0	0	0	18	
152	6478/60	32	F	+	+	0	0	0	-	-	-	0	0	0	0	0	

Case no	I	17	18	19	20	21	22	23	24	25	26	27	Clearances ml min/1.73 m BSA				Intercurrent diseases	Diagnosis	Verification Ht - Weight	O - Oper
													PAH	Inulin	Endog creatinine	General sign of disease				
		Rectal r.s.	Clo dy III small no urine	Pregos ccs (no)	Phenacilin atyde	Pre ious bladder catheteria	BP (mm Hg) 24 hours (max) or on admission	BP (mm Hg) 4 hours (m n)	Ocular fundi (Keith & Wagener) FHT	Serum creatinine (mg/100 ml)	Water loading test (mOsmol/lit)	Prothrombin								

GROUP B2 Renal disease (except pyelonephritis) with unilateral or mainly asymmetric involvement

106	0	0	0	0	0	0	115/95	95/60	0	1.0	-	0	(608)	(118)	(104)	+	Art. hypert.	11	0
107	0	0	0	0	0	0	100/120	150/100	0	1.2	-	0	(390)	(177)	(80)	+	Art. hypert.	4	0
108	0	0	0	0	0	0	140/85	100/65	1 H	1.6	25	(+)	(274)	(79)	(70)	+	Art. hypert. nephrol th.	3	0
109	0	0	0	0	0	0	175/70	-	1 H	1.6	215	(+)	-	-	-	+	Art. hypert.	3	0
110	+	0	0	0	0	0	140/80	-	-	1.3	-	(+)	-	-	(97)	+	Art. hypert.	6	0
111	0	0	0	0	0	0	145/90	155/75	1 H	1.2	-	0	(531)	(60)	(65)	+	Art. hypert.	7	0
112	0	0	0	0	0	0	190/100	135/90	1 H	1.0	115	0	(411)	(88)	(47)	+	Art. hypert.	8	0
113	0	0	0	0	0	0	210/110	180/95	1 H	1.1	210	0	293	-	88	+	Art. hypert.	9	0
114	0	0	0	0	0	0	180/95	110/70	0	1.1	210	0	338	-	113	+	Art. hypert.	10	0
115	0	+	0	0	0	0	115/80	105/60	0	1.2	140	(+)	638	-	93	+	Art. hypert.	11	0
116a	0	0	0	0	0	0	170/85	105/70	0	1.1	-	0	638	-	105	+	Art. hypert.	12	0
116b	0	0	0	0	0	0	170/85	105/70	0	1.1	-	0	46	-	105	+	Art. hypert.	13	0

GROUP B3 Arterial hypertension with stenosis or aneurysm of the main renal artery

117a			III		-	215/135	145/70	1	1.4		+	+	+	-	-	-	+		11	O
117b						220/115	180/105	1	1.3		0	-	-	-	-	-	+		12	O
118	0	0	I	0		240/150	200/130	1 (H)	1.4		-	-	-	(280)	(88)	(47)	+	Hypoplas. R. kidney	13	O
119					0	170/105	150/80	II	1.2		+	-	-	-	-	-	+		14	O
120			0	-	+	270/140	150/80	II (H)	1.0		(+)	-	-	-	-	-	+		15	O
121					0	160/140	230/130	II	1.5		0	-	-	-	-	-	+		16	O
122	0	0	I	0	0	220/105	170/100	II	1.1		(+)	303	-	-	-	76	+		17	O
123	0	0	III	-	+	210/100	140/65	II	0.8		0	-	-	-	-	-	+		18	O
124					0	175/110	120/85	II	1.4		-	-	-	-	-	-	+		19	O
125					0	185/105	165/70	-	-		0	-	-	-	-	-	+		20	O
126			II		-	200/100	160/85	0	1.0		0	-	-	-	-	-	+		21	O
127					+	190/170	140/60	0	1.0		0	-	-	-	-	-	+		22	O
128					0	255/120	180/100	II (III)	1.6		+	184	-	-	-	6	+		23	O
129			II		0	245/120	140/100	II	1.0		0	-	-	-	-	-	+		24	O
130	0	0	0	0	0	265/140	200/170	III III	0		0	-	-	-	-	38	+		25	O
131	0	0	(-)	0	0	240/145	205/100	III	1.0		(+)	-	-	-	-	47	+		26	O

GROUP B4 Renal disease with expected homogeneous involvement of both kidneys

132	0	0	0	0	0	0	200/120	155/100	0	1.4	-	0	-	-	-	0	Art. hypert.	11	0
133	0	0	0	0	0	0	140/110	125/90	1	1.0	-	0	-	-	-	+	Art. hypert.	12	0
134	0	0	0	0	0	0	150/110	180/170	1 H	1.0	-	0	-	-	-	+	Art. hypert.	13	0
135	0	0	0	0	0	0	200/175	160/100	1	0.9	70	0	417	-	83	+	Art. hypert.	14	0
136	0	0	0	0	0	0	160/95	125/75	0	1.2	217	+	487	-	84	+	Art. hypert.	15	0
137	0	0	0	0	0	0	140/100	-	0	1.4	-	+	(410)	-	(65)	+	Art. hypert.	16	0
138a	0	0	0	0	0	0	140/90	125/75	0	1.7	120	+	(150)	-	(21)	+	Art. hypert.	17	0
138b	0	0	0	0	0	0	220/140	180/115	1 H	1.1	-	+	-	-	-	-	Art. hypert.	18	0
139	0	0	0	0	0	0	130/80	105/60	0	1.2	65	+	(337)	-	(63)	+	Art. hypert.	19	0
140	0	0	0	0	0	0	135/65	90/65	0	1.3	-	+	(308)	-	(71)	+	Art. hypert.	20	0
141	0	0	0	0	0	0	145/95	130/75	0	1.8	-	+	-	-	-	-	Art. hypert.	21	0
142	0	0	0	0	0	0	190/140	145/75	1 H	1.2	158	+	(563)	-	(113)	+	Art. hypert.	22	0
143	0	0	0	0	0	0	170/95	135/80	0	1.4	-	+	491	-	104	+	Art. hypert.	23	0
144	0	0	0	0	0	0	140/80	105/55	0	1.2	160	+	537	-	94	+	Art. hypert.	24	0
145	0	0	0	0	0	0	145/110	140/90	1 H	1.1	-	+	110	-	27	+	Art. hypert.	25	0
146	0	0	0	0	0	0	150/105	115/80	0	1.7	-	+	191	-	49	+	Art. hypert.	26	0
147	0	0	0	0	0	0	130/90	110/70	0	1.9	160	+	-	-	66	+	Art. hypert.	27	0

GROUP B5 Patients with symptoms from the lower urinary tract in whom involvement of the kidneys could not be ruled out

148	0	0	0	0	0	0	135/75	90/65	0	1.8	-	+	(577)	-	(177)	+	Art. hypert.	11	0
149	0	0	0	0	0	0	115/70	95/55	0	1.4	145	+	366	-	(37)	+	Art. hypert.	12	0
150	0	0	0	0	0	0	170/75	94/60	0	1.0	85	+	(6.4)	-	(88)	+	Art. hypert.	13	0
151	0	0	0	0	0	0	120/75	90/60	0	0.8	90	+	-	-	-	-	Art. hypert.	14	0
152	0	0	0	0	0	0	75/5	90/60	0	1.0	85	+	413	-	144	+	Art. hypert.	15	0

Hypoplas. R. kidney H. dilat. L. kidney Chronic glom. nephrit. art. sten. R. main renal art. Sten. R. main renal art. Sten. R. main renal art. No.

H. dilat. R. kidney H. dilat. L. kidney Chronic glom. nephrit. art. sten. main renal art. Sten. R. main renal art. Sten. R. main renal art. No.

H. dilat. R. kidney H. dilat. L. kidney Chronic glom. nephrit. art. sten. main renal art. Sten. R. main renal art. Sten. R. main renal art. No.

H. dilat. R. kidney H. dilat. L. kidney Chronic glom. nephrit. art. sten. main renal art. Sten. R. main renal art. Sten. R. main renal art. No.

Table IVb (continued)

Case no	Case Record no	Age at invest (yrs)	Sex	Cardinal criteria of pyelonephritis										Histologic exam B = Biopsy R = Resection or ectomy A = Autopsy	Bacteriuria no fms	Urinary sediment	Renal exam	No. post mortem/No. criteria tested
				Cystitis without fever	Cystitis with fever	Chills without cystitis	Hist. of loin pain	Loin tenderness to palpation	Acidif. abt (pH)	Max. conc. ability (mOsmol/kg)								
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16			
GROUP B2 Renal disease (except pyelonephritis) with unilateral or mainly asymmetric involvement																		
106	4039/61	34	F	0	0	0	+	0	—	973	—	0	0	0	16			
107	3799/65	18	F	0	0	0	—	0	5.0	—	—	0	0	0	17			
108	8562/59	36	F	0	0	+	+	0	4.91	535	B +	160 ml Ec	0	0	48			
109	8695/60	52	M	0	0	—	—	0	—	—	—	—	0	0	05			
110	6381/61	27	M	—	—	—	—	0	—	—	—	50 000 Ec	+	0	14			
111	9940/58	62	M	0	0	0	0	0	5.05	310	R 0	0	0	0	16			
112	6149/59	37	M	0	0	0	0	0	—	—	R +	0	0	0	16			
113	7373/60	61	F	0	0	0	0	0	6.04	737	B +	6 ml Ec	+	+	68			
114	1738/63	55	F	+	0	0	+	0	6.23	930	—	600 000 S alb	+	+	17			
115	5574/62	36	F	0	0	+	+	+	6.14	300	B 0 x	20 ml Aa	+	+	68			
116a	3227/61	17	F	0	0	0	0	+	—	—	B 0 x	0	+	+	68			
116b		18	F	0	+	0	0	+	4.84	1060	B +	0	0	0	38			
GROUP B3 Arterial hypertension with stenosis or aneurysm of the main renal artery																		
117a	3533/62	55	F	+	0	0	0	(+)	—	—	—	0	0	0	15			
117b	3532/62	56	F	0	0	0	0	0	—	—	—	0	0	0	06			
118	3794/58	30	F	0	0	0	0	0	—	—	R 0 x	0	0	0	06			
119	1069/62	66	M	0	0	0	0	0	—	—	—	0	0	0	05			
120	7084/61	33	F	0	0	0	0	0	10.74	—	B 0 x	0	+	0	17			
121	2765/60	52	M	0	0	0	0	0	—	1025	—	0	0	0	06			
122	† 8361/61	36	F	+	0	0	0	(+)	—	—	A —	0	+	0	05			
123	6008/62	61	M	0	0	0	0	0	—	—	—	0	0	0	05			
124	1282/62	53	M	0	0	0	0	0	—	—	—	0	0	0	05			
125	671/62	68	M	0	0	0	0	0	—	—	—	0	0	0	05			
126	411/62	63	F	0	0	0	0	0	—	—	—	0	0	0	04			
127	9186/61	64	F	0	0	0	0	0	—	—	—	0	0	0	05			
128	3803/62	45	M	0	0	0	0	0	—	—	—	0	0	0	05			
129	6358/62	54	F	+	0	0	0	0	—	—	—	0	0	0	05			
130	501/63	49	F	0	0	0	0	0	—	—	—	0	0	0	05			
131	3395/61	57	F	(+)	0	0	0	0	—	—	—	0	0	0	05			
GROUP B4 Renal disease with expected homogeneous involvement of both kidneys																		
132	2766/62	53	M	0	0	0	0	0	—	—	—	0	0	0	05			
133	5965/62	31	F	+	+	0	0	0	—	—	—	0	0	0	15			
134	† 1524/61	33	M	0	0	0	0	0	—	407	A —	0	0	0	17			
135	2601/61	46	F	(+)	0	0	0	0	—	695	B 0 x	~100 000 Pv	0	0	17			
136	4667/58	48	M	0	0	0	(+)	0	5.2	484	R 0 x	0	0	0	18			
137	† 7143/58	23	M	0	0	0	0	0	—	745	A 0 x	0	0	0	15			
138a	† 1198/60	33	F	0	0	0	+	0	—	565	R 0 x	0	0	0	16			
138b		35	F	0	0	0	0	0	—	—	A 0 x	0	0	0	7			
139	9432/59	35	F	+	+	0	+	0	5.4	—	B 0	0	0	0	06			
140	2715/62	19	F	0	0	0	0	0	—	—	B 0	0	0	0	06			
141	24/765	42	F	0	0	0	0	0	—	—	B 0 x	0	0	0	06			
142	733/60	44	M	(+)	0	0	0	0	4.95	688	B 0 x	0	0	0	16			
143	† 4454/62	28	M	0	0	0	0	0	—	—	B A 0 x	0	0	0	06			
144	3703/60	14	F	0	0	0	0	0	5.0	835	B 0 x	4 ml Aa	0	0	16			
145	5058/64	39	F	0	0	+	+	0	—	318	B 0 x	17 ml Ec	0	0	7			
146	6307/65	31	M	0	0	0	0	0	—	3.0	B 0	0	0	0	38			
147	8963/60	35	F	0	0	0	0	0	5.4	380	B 0 x	0	0	0	38			
GROUP B5 Patients with symptoms from the lower urinary tract in whom involvement of the kidneys could not be ruled out																		
148	8890/58	37	F	0	0	0	+	0	—	875	B 0	0	0	0	15			
149	† 110/59	41	F	+	0	0	+	0	4.9	8.5	B ()	0	0	0	17			
150	8825/60	40	F	+	0	0	0	0	—	1130	B 0	0	0	0	17			
151	3971/60	21	F	+	+	0	0	0	5.10	1000	B 0	400 ml Ec	0	0	7			
152	6418/60	32	F	+	+	0	0	0	—	—	—	0	0	0	17			

Table V Comparison between clinical and histologic observations in 82 cases of pyelonephritis (chronic or acute uncomplicated or complicated groups A1 A2 B1)

	Total no of cases			Agreeing clinical diagnosis						Doubt ful		Not agreeing clinical diagnosis					
Group	A1	A2	B1	A1	A2	B1	A1	A2	B1	A1	A2	B1	A1	A2	B1		
	No	No	No	No	%	No	/	No	/	No	%	No	%	No	/	No	
Specimen obtained at needle biopsy	52	9	12	34	65	2	22	12	100	2	4	16	31	7	78		
Specimen obtained at surgery or autopsy	4	—	5	4	100	—	—	5	100	—	—	—	—	—	—	—	
Sum	61			57 = 70 %						2 = 2 %		23 = 28 %					

noting it as interstitial nephritis. Moreover he pointed out that the criteria of Weiss & Parker (267) — periglomerular fibrosis, interstitial cell infiltration, thyroid like tissue — are not pathognomonic to chronic pyelonephritis. These features are also seen in patients with e.g. renal disease due to vascular lesions or with renal damage caused by toxic or other exogenous agents (e.g. phenacetin abuse).

Thus it is not possible to set up either clinical or histologic criteria which must always be fulfilled for a diagnosis of chronic pyelonephritis. In the present study I have nevertheless included the histologic examination in the group of cardinal criteria since the finding of interstitial nephritis at this examination does when other criteria are fulfilled strengthen the suspicions of chronic pyelonephritis.

The value of the histologic examination for the diagnosis is illustrated in Table V. It shows that in about 70 per cent of the 82 cases of chronic or acute pyelonephritis — of both uncomplicated and complicated type — in which this examination was made the histologic features largely corresponded

to the clinical symptoms and signs (at least three cardinal criteria). In 2 per cent the examiner was doubtful and in 28 per cent the microscopic features did not correspond to the clinical features. Similar conditions were reported by Brod (29).

Brun & Raaschou (38-39) found no other clinical or histologic differences between patients who had consumed phenacetin and those who had not than the presence in some of the former cases of necrotic tissue in the biopsy specimen or a necrotic papilla in the urine. Differences may also be observed at autopsy. This agrees with my experience. However Ringertz (211) like Spühler & Zollinger (239) stated that there are additional histologic lesions which distinguish chronic interstitial nephritis — such as occurs in abuse of phenacetin — from chronic pyelonephritis originating in the renal pelvis. For further discussion of this question, reference is made to Hultgren (110).

6. Bacteriuria

Since pyelonephritis presupposes the existence of renal infection demonstration of bacteriuria was naturally of the greatest im-

series of patients with chronic pyelonephritis that 45 per cent complained of dysuria and pollakiuria

2 *Tenderness to palpation*

On examination of the patients, particular attention was focused on the existence of tenderness to palpation over one or both loins. Although this may occur in the presence of, e.g. polycystic kidneys or a renal tumour, it strongly supported the diagnosis when chronic pyelonephritis was suspected. Brod (29) stated that in those who complained of pain in the loins (64 per cent of the material) tenderness to palpation was present.

3 *Ammonium chloride load*

Decreased ability to acidify the urine is an important diagnostic sign in chronic pyelonephritis (77, 135) and in other renal diseases (10b, 272, 273).

The technique used in the ammonium chloride load test is described in Chapter VII. It was not performed in patients with a tendency to acidosis nor in those with urea splitting bacteria in the urine. The limit of normal renal acidifying ability was given by Bengtsson (10b) as pH 5.2, and by Wrong (272, 273) as pH 5.5. I took pH 5.3 (18) as the normal limit on the basis of 11 determinations in 9 healthy subjects aged 23—54 years.

4 *Renal concentration ability*

This was determined in mOsmol/lit. The test was made after the patient had been under observation in hospital for a few days and had been given a standard diet.

Like Bengtsson (10b) I set the limit of normal osmolality at 800 mOsmol/lit irrespective of age, even though it has been

shown that the osmolality of the urine starts to decrease as early as 30—40 years of age (151, 264). The performance of the test is described in Chapter VII.

5 *Histologic changes*

Renal biopsy, in which one is fortunate enough to obtain a specimen of tissue with pyelonephritic lesions is, naturally, of great value for the diagnosis. A biopsy specimen is often difficult to judge, particularly if it consists only of medullary tissue and does not contain any glomerulus. Obviously the results were more reliable when the microscopic examination was made on a specimen of resected tissue, or post mortem. The nature of the specimen on which the histologic diagnosis was based is shown in Table IV. In the present study, it was of the utmost value that all the specimens were examined by the same pathologist. Even if a biopsy specimen did not exhibit interstitial lesions this was not considered to argue against the diagnosis, since the specimen might have been taken from intact renal tissue, which is often present between the focal lesions.

The histologic criteria of pyelonephritis were those set up by Brun & Raaschou (39) *ie*,

Cell casts

Interstitial cell infiltration
Invasive glomerulitis
Periglomerular fibrosis
Tubular atrophy
Peritubular fibrosis
Dilated tubules with acellular casts
(thyroid like)

Brun (36b) has however stated that in recent years he has not made a diagnosis of chronic pyelonephritis on a histologic specimen but has confined himself to de

Table V Comparison between clinical and histologic observations in 82 cases of pyelonephritis (chronic or acute — uncomplicated or complicated groups A1 A2 B1)

Group	Total no of cases			Agreeing clinical diagnosis						Doubtful		Not agreeing clinical diagnosis					
	A1	A2	B1	A1	A2	B1	A1	A2	B1	A1	A2	B1	A1	A2	B1		
	No	No	No	No	/	No	/	No	No	%	No	%	No	%	No	%	
Specimen obtained at needle biopsy	52	9	12	34	65	2	22	12	100	2	4	16	31	7	78		
Specimen obtained at surgery or autopsy	4		5	4	100	—		5	100							—	—
Sum	III			57 = 70 %						2 = 2 %		23 28 /					

noting that interstitial nephritis. Moreover he pointed out that the criteria of Weiss & Parker (267) — per glomerular fibrosis interstitial cell infiltration thyroid like tissue — are not pathognomonic to chronic pyelonephritis. These features are also seen in patients with e.g. renal disease due to vascular lesions or with renal damage caused by toxic or other exogenous agents (e.g. phenacetin abuse).

Thus it is not possible to set up either clinical or histologic criteria which must always be fulfilled for a diagnosis of chronic pyelonephritis. In the present study I have nevertheless included the histologic examination in the group of cardinal criteria since the finding of interstitial nephritis at this examination does when other criteria are fulfilled strengthen the suspicions of chronic pyelonephritis.

The value of the histologic examination to the diagnosis is illustrated in Table V. It shows that in about 70 per cent of the 8 cases of chronic or acute pyelonephritis of both uncomplicated and complicated type — in which this examination was made the histologic features largely corresponded

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6. Bacteriuria

Since pyelonephritis presupposes the existence of renal infection demonstration of bacteriuria was naturally of the greatest im-

Table VIa Survey of the cardinal diagnostic criteria in chronic non-obstructive pyelonephritis (group

Criterion	No of invest	Limit	Positive		Negative		D
			No	%	No	%	No
Cystitis without fever	66		33	50	33	50	
Cystitis with fever	68		46	68	20	29	2
Chills without cystitis	63		15	24	47	75	1
Hist of loin pain	69		48	70	17	25	4
Loin tenderness to palpation	69		29	42	40	58	
Acidif abil (pH)	60	pH 5.3	30	50	30	50	
Max conc ability (mOsmol/lit)	66	800 mOsmol/lit	57	86	9	14	
Histologic exam (cf Table V)	56		38	68	16	29	2
Bacteriuria (no/ml)	69	100 000 bact/ml	63	91	6	9	
Urinary sediment	69	10 WBC/high power field	60	87	9	13	
Roentgenol exam	68		46	68	22	32	

portance This applied despite the fact that the urine may be sterile even in unquestionable pyelonephritis, as a result either of a non functioning kidney or of anti infective therapy

A thorough bacteriologic investigation was made in all the patients in the present series It included quantitative determination of the bacteria, and determination of the sensitivity to all the common chemotherapeutics and antibiotics (see Chap VII) When e.g., *Staphylococcus albus* and diphtheroids were present separately or together on a few occasions, and then only in small numbers they were regarded as apathogenic and were not counted as a positive diagnostic criterion

A bacterial count was made and the value obtained was taken into account irrespective of whether or not the bacteriuria had given rise to symptoms Asymptomatic bacteriuria is considered to occur in about 4 per cent of healthy individuals who have not been hospitalized (128—130) and in up to 30 per cent of patients with severe intestinal diseases (147) and women with diabetes mellitus (127 237)

In accordance with Kass (128) I took

100,000 bacteria/ml as the lower limit of significant bacteriuria in specimens from the urinary bladder The corresponding figure for urine obtained by ureteric catheterization was 10,000 bacteria/ml (127) In the few cases where no bacterial count was made, bacteriuria was denoted as copious (corresponding to > 100 000 bact/ml), moderate (corresponding to 10,000—100,000/ml) or sparse (100—10,000/ml)

7 Urinary sediment

Ten or more leukocytes per high power field in the spun sediment were defined as definite pyuria In many cases, I was able to confirm that the sediment may be negative in patients with pyelonephritis despite bacteriuria I considered the most important findings in the sediment in pyelonephritis to be pyuria and the discrepancy between the numerous leukocytes and the few red cells Granulated cylinders were often present consisting of degenerated white blood cells and tubular epithelium as a sign of involvement of renal tissue (206) A feature regarded as especially important for the diagnosis was the demon

Table VIb Survey of the cardinal diagnostic criteria in suspected chronic or acute non-obstructive pyelonephritis (group A2)

Criterion	No of invest	Limit	Positive		Negative		Doubtful	
			No	%	No	%	No	%
Cystitis without fever	11		11	55	4	36	1	9
Cystitis with fever	11		5	45	6	55		
Chills without cystitis	11		1	9	10	91		
Hist of loin pain	10		4	40	5	50	1	10
Lo n tenderness to palpation	11				11	100		
Acidif abil (pH)	8	pH 5.3	2	25	11	75		
Max conc ability (mOsmol/lit)	11	800 mOsmol/lit	1	9	10	91		
Histologic exam (cf Table V)	9		2	22	7	78		
Bacteriuria (no /ml)	11	100 000 bact/ml	4	36	7	64		
Urinary sediment	11	10 WBC/high power field	3	27	8	73		
Roentgenol exam	11				11	100		

stration by Sternheimer & Malbin staining (243) of the characteristic vacuolated cells (85) Renal epithelium was often present

The aforementioned features as well as demonstration of bacteria in centrifuged specimens were regarded as positive findings

8 Roentgenologic changes

Excretory pyelography Dejdar (62) and Dejdar & Prat (63) stressed the value of roentgenologic examination of the kidneys in chronic pyelonephritis partly to demonstrate any asymmetric involvement (cf Chap I) In the present series the roentgenologic diagnosis of chronic pyelonephritis was based on the criteria of Olsson (182) and that of renal papillary necrosis on the criteria of Lindvall (153)

Retrograde pyelography was only occasionally performed It was done either because renal function was greatly impaired or because one kidney was silent for some other reason

Selective renal angiography was performed in hypertensive patients with chronic pyelonephritis when arterial hypertension was

suspected to be caused by stenosis of the main renal artery It was also done in the absence of hypertension when inflammatory foci or scarring were suspected

Urethrocytography On technical grounds this was carried out in only a few patients

A survey of the cardinal diagnostic criteria and the number and incidence of cases of chronic and acute non obstructive pyelonephritis in which they were fulfilled is given in Table VI together with the total number of investigations

B General Symptoms of Renal Disease

Patient's statements

The patient's statements about his complaints are naturally of great importance for the diagnosis This applies irrespective of whether or not he ascribes them to renal disease Obviously when such symptoms were mentioned or discussed due reservations had to be made for the unreliability of the patient's statements

Table VIa Survey of the cardinal diagnostic criteria in chronic non-obstructive pyelonephritis (group A)

Criterion	No of invest	Limit	Positive		Negative		Doubt
			No	%	No	%	
Cystitis without fever	66		33	50	33	50	
Cystitis with fever	68		46	68	20	29	2
Chills without cystitis	63		15	24	47	75	1
Dist of loin pain	69		48	70	17	25	4
Loin tenderness to palpation	69		29	42	40	58	
Acidif abil (pH)	60	pH 5.3	30	50	30	50	
Max conc ability (mOsmol/lit)	66	800 mOsmol/lit	57	88	9	14	
Histologic exam (cf Table V)	56		38	68	16	29	2
Bacteriuria (no./ml)	69	100 000 bact/ml	63	91	6	9	
Urinary sediment	69	10 WBC/high power field	60	87	9	13	
Roentgenol exam	68		46	68	22	32	

portance This applied despite the fact that the urine may be sterile even in unquestionable pyelonephritis, as a result either of a non functioning kidney, or of anti infective therapy

A thorough bacteriologic investigation was made in all the patients in the present series It included quantitative determination of the bacteria, and determination of the sensitivity to all the common chemotherapeutics and antibiotics (see Chap VII) When *eg*, *Staphylococcus albus* and diphtheroids were present separately or together on a few occasions and then only in small numbers, they were regarded as apathogenic and were not counted as a positive diagnostic criterion

A bacterial count was made and the value obtained was taken into account, irrespective of whether or not the bacteriuria had given rise to symptoms Asymptomatic bacteriuria is considered to occur in about 4 per cent of healthy individuals who have not been hospitalized (128—130) and in up to 30 per cent of patients with severe intestinal diseases (147) and women with diabetes mellitus (127—257)

In accordance with Kass (128) I took

100 000 bacteria/ml as the lower limit of significant bacteriuria in specimens from the urinary bladder The corresponding figure for urine obtained by ureteric catheterization was 10,000 bacteria/ml (127) In the few cases where no bacterial count was made bacteriuria was denoted as copious (corresponding to > 100,000 bact/ml) moderate (corresponding to 10,000—100,000/ml) or sparse (100—10,000/ml)

7 Urinary sediment

Ten or more leukocytes per high power field in the spun sediment were defined as definite pyuria In many cases I was able to confirm that the sediment may be negative in patients with pyelonephritis, despite bacteriuria I considered the most important findings in the sediment in pyelonephritis to be pyuria and the discrepancy between the numerous leukocytes and the few red cells Granulated cylinders were often present consisting of degenerated white blood cells and tubular epithelium, as a sign of involvement of renal tissue (206) A feature regarded as especially important for the diagnosis was the demon

Table VIb *Survey of the cardinal diagnostic criteria in suspected chronic or acute non-obstructive pyelonephritis (group A2)*

Criterion	No of invest	Limit	Positive		Negative		Doubtful	
			No	%	No	%	No	%
Cystitis without fever	11		6	55	4	36	1	9
Cystitis with fever	11		5	45	6	55		
Chills without cystitis	11		1	9	10	91		
Hist. of loin pain	10		4	40	5	50	1	10
Lo n tenderness to palpation	11				11	100		
Acidif abil (pH)	8	pH 5.3	2	25	6	75		
Max conc ability (mOsmol/lit)	11	800 mOsmol/lit	1	9	10	91		
Histologic exam (cf Table V)	9		2	22	7	78		
Bacteremia (no/ml)	11	100 000 bact/ml	4	36	7	64		
Urinary sediment	11	III WBC/high power field	3	27	8	73		
Roentgenol exam.	11				11	100		

stration by Sternheimer & Malbin staining (243) of the characteristic vacuolated cells (85). Renal epithelium was often present.

The aforementioned features as well as demonstration of bacteria in centrifuged specimens were regarded as positive findings.

8 Roentgenologic changes

Excretory pyelography: Dejdar (62) and Dejdar & Prat (63) stressed the value of roentgenologic examination of the kidneys in chronic pyelonephritis partly to demonstrate any asymmetric involvement (cf Chap I). In the present series the roentgenologic diagnosis of chronic pyelonephritis was based on the criteria of Olsson (182) and that of renal papillary necrosis on the criteria of Lindvall (153).

Retrograde pyelography was only occasionally performed. It was done either because renal function was greatly impaired or because one kidney was silent for some other reason.

Selective renal angiography was performed in hypertensive patients with chronic pyelonephritis when arterial hypertension was

suspected to be caused by stenosis of the main renal artery. It was also done in the absence of hypertension when inflammatory foci or scarring were suspected.

Urethrocystography. On technical grounds this was carried out in only a few patients.

A survey of the cardinal diagnostic criteria and the number and incidence of cases of chronic and acute non obstructive pyelonephritis in which they were fulfilled is given in Table VI together with the total number of investigations.

II General Symptoms of Renal Disease

Patient's statements

The patient's statements about his complaints are naturally of great importance for the diagnosis. This applies irrespective of whether or not he ascribes them to renal disease. Obviously when such symptoms were mentioned or discussed due reservations had to be made for the unreliability of the patient's statements.

Reasons for consultation

All patients investigated and treated at the Renal Clinic, St Erks Sjukhus, either as out patients or in patients, were referred by other physicians, who had treated them for a varying period. For this reason it was sometimes difficult to reconstruct the cause of their originally having consulted a physician. However, whenever possible, the first symptom was noted.

Duration of complaints

It was often hard to obtain any exact idea of the duration of the urinary tract symptoms. This was due partly to the chronicity of the disease with alternating remissions and exacerbations. Another contributory factor was that the onset was frequently insidious or completely asymptomatic. In some patients, it took place already in childhood. This was important information but was sometimes difficult to elicit. In view of the difficulty of determining the duration of the symptoms in the individual case this information has been omitted from Table IV.

Haematuria

Even though macroscopic haematuria is more common in other forms of renal disease it may occur in chronic pyelonephritis and be noticed by the patient. As a rule, it is to be ascribed to passage of a concretion or a renal papilla, or to a mucosal lesion which, in turn is caused by infection.

Appearance and smell of urine

Cloudy urine with a bad smell is obviously an important symptom which may lead to suspicions of urinary tract disease. The patient not infrequently sought medical advice for this reason, and investigation — includ-

ing microscopic examination of the sediment, bacterial culture and renal function tests — sometimes disclosed the existence of an otherwise asymptomatic chronic infection, possibly pyelonephritis. Often, the patient noticed that passage of such urine was combined with back pain and possibly with a rise in temperature. A history of such symptoms naturally strengthened the suspicions of chronic pyelonephritis.

Influence of pregnancy

Attacks of acute pyelonephritis are known to be more frequent in pregnant women than in others. Moreover, numerous investigations have shown that asymptomatic bacteriuria often occurs in pregnancy (49, 78, 103, 124, 125, 128, 129, 133, 146, 174, 190, 254) and that the incidence is higher than in non pregnant women. It is also important to bear in mind that asymptomatic bacteriuria in pregnancy leads to clinically demonstrable pyelonephritis in 35—40 per cent of cases (129).

I considered it of interest to ascertain whether the patient's bladder had been emptied by catheterization in connexion with childbirth. This was because such a procedure may cause infection of the lower urinary tract and thereby gradually lead to pyelonephritis. The number of childbirths is therefore listed in Table IV as well as the minimum number of associated catheterizations of the bladder.

Phenacetin abuse

It was extremely difficult to obtain any exact information about the amount of phenacetin (acetophenetidine) taken. The patients adopted an evasive attitude when questioned on this matter. They not infrequently underestimated their real consumption.

tion by conscious forgetfulness. In view of the unreliability of these statements I did not wish to apply Schweingrube's (225) limit for abuse *i.e.* at least 1 g of phenacetin per day for more than 1 year or Bengtsson's (10b) lower limit of at least 0.3 g/day for at least 1 year. Instead I denoted as abuse a regular preferably daily intake of phenacetin containing preparations for a long time (at least 1 year).

Most authors now agree that a relation exists between phenacetin abuse and renal papillary necrosis (10b 11 88 91 177 239) whereas others contend that additional substances — *e.g.* acetylsalicylic acid — may be able to cause interstitial nephritis (244). I shall not enter into a detailed discussion of the relation between phenacetin abuse and chronic pyelonephritis but refer to the abundant literature on the subject.

In the present series the coexistence of chronic pyelonephritis and renal papillary necrosis is recorded in the tables but no attempt was made to distinguish between these cases and pure pyelonephritis as regards renal function. This is because papillary necrosis without concomitant urinary tract infection and signs of chronic pyelonephritis was demonstrable in only two patients (cases 146 147).

Previous instrumentation

Since introduction of an instrument into the urinary tract may produce infection the patients were always questioned on this matter.

C. General Signs of Renal Disease

Physical examination

The physical findings characteristic of *uraemia* and *oedema* are too well known to require any description.

Blood pressure and fundus changes

It cannot be ruled out that arterial hypertension may cause secondary more severe renal damage and thus mask any inappreciable asymmetric involvement (*cf.* Chap. X p. 105). For this reason the arterial blood pressure is noted in the tables.

For the same reason the optic fundi were inspected and any changes observed were divided into groups according to the classification of Keith & Wagener (*cf.* Chap. VII). Ophthalmoscopy was performed by an ophthalmologist.

Serum creatinine

The serum creatinine was recorded as a test of renal function; this determination is made routinely at the Central Laboratory of our hospital. The test is comparable with the determination of BUN and NPN (118).

Water loading test of Volhard

This test was made in every patient. In individuals with healthy kidneys can as a rule dilute the urine to an osmolality of 50 mOsmol/lit or even lower. However as far as I have been able to ascertain no investigation has been made to determine the dilution that is to be required if the value is not to be regarded as a sign of reduced renal dilution ability. In our experience at the Renal Clinic this test is of little clinical worth in the diagnosis of renal diseases.

Proteinuria

In chronic pyelonephritis protein is not usually present in the urine in any great quantity *i.e.* seldom in excess of 5 g/24 hours (29). I included this test because heavy proteinuria is more characteristic of other renal diseases than of pyelonephritis. As far as possible I tried to determine the 24 hour excretion.

It cannot be ruled out that, in a few cases, small quantities of vaginal secretion contributed to a positive protein reaction. This source of error was generally eliminated by the technique of taking the sample with a glass tube (cf Chap VII)

Creatinine clearance

The *endogenous creatinine clearance* was determined in almost every case

Inulin clearance

On practical grounds the inulin clearance was determined only in those patients who underwent renal vein catheterization. In the other cases, the endogenous creatinine clearance was determined instead, even if this is known to be an unreliable measure of the glomerular filtration rate (232)

PAH clearance

Since most of the patients in my series had decreased PAH extraction as a sign of renal damage, the PAH clearance is of little value as an expression of the renal blood flow. The values were nevertheless included, to illustrate the use of this parameter as a diagnostic test in chronic pyelonephritis

D General Signs of Disease

This group includes such vague unspecific symptoms as fatigue, headache, weight loss and dyspnoea, but no urinary tract symptoms. Instead the gastrointestinal symptoms dominate, e.g. nausea and sometimes vom-

iting and other digestive or intestinal complaints

Brod (28) found such unspecific symptoms in $\frac{1}{4}$ to $\frac{1}{3}$ of his patients, and similar figures have been reported by other workers (135). The patients in my series were questioned about such symptoms and examined for their presence, but they were not analyzed in any detail. This is because, as already mentioned, they are not significant either for renal disease in general or for chronic pyelonephritis in particular. Such symptoms are common, but did not occur consistently in my cases. In Table IV, + denotes their occurrence, and 0 that they were absent.

In every case the ECG was recorded and a roentgenologic examination was made of the heart and lungs. In addition the following determinations were made: haemoglobin in blood, red cell count, leukocyte count and differential leukocyte count, ESR, serum chlorides, sodium, potassium, phosphorus, calcium and alkali reserve. The results of these investigations are given and commented on only when there is special reason to do so.

The diagnostic observations listed in Table IV include the finding of any complicating disease of the kidneys and/or lower urinary tract as well as other complicating diseases which might have influenced the results of the tests. Particular attention was focused on diabetes mellitus, since a relation has been stated to exist between this disease and chronic pyelonephritis (127, 257). My series contained only one diabetic patient (case 30).

CHAPTER IV

DIAGNOSIS OF OTHER RENAL DISEASES

In addition to the cases of chronic pyelonephritis the present series contains some patients with other renal diseases. The chief reason for including them was that they were regarded to be suitable as comparative material. Since the diagnosis seldom presented any difficulties in these cases a discussion of the diagnostic criteria is superfluous. All the investigations made in the diagnosis of chronic pyelonephritis were used in these cases also. Consequently only brief mention will be made of the most important investigations apart from the fundamental ones.

In *arterial hypertension* in which stenosis of the main renal artery was suspected renal angiography was needed to verify the diagnosis. The urinary catecholamines were determined in all hypertensive patients to rule out the existence of phaeochromocytoma.

In *chronic glomerulonephritis* and the *nephrotic syndrome* the customary urine and renal function tests were complemented by determination of the total serum protein, electrophoresis of serum proteins and the antistreptolysin and antistaphylococcal titres in serum.

In *polycystic kidney* various roentgenologic methods were used as diagnostic aids.

Suspensions of *renal amyloidosis* could be

verified in some cases by examination of a renal biopsy specimen.

In two cases of *renal tuberculosis* with complicating chronic pyelonephritis a definite diagnosis was reached with the help of culture in Löwenstein medium and guinea pig inoculation.

When *residual urine* was suspected to be present this was determined in the customary way.

If *hyperparathyroidism* was suspected repeated determinations were made of the calcium and phosphorus in serum and urine. This was complemented by roentgenologic examinations with the object of demonstrating the characteristic periosteal erosions and bone cysts.

It need scarcely be pointed out that all the methods of examination described do not form part of the routine battery of tests used for diagnosis at the Renal Clinic. All of them are unquestionably of diagnostic value in certain doubtful cases but in the majority of cases of renal disease the diagnosis can be established with satisfactory certainty without having to resort to such methods as e.g. renal vein catheterization and ureteric catheterization. This question is discussed in detail in Chapter V (p. 112).

CASE MATERIAL

The case material on which the present investigation is based consists of totally 152 patients investigated and treated at the Central Clinical Laboratory and the Renal Clinic in 1958—1963 inclusively. All the patients had been hospitalized at least once for investigation and, at the time of writing, most of them are still under regular control in the out patient department. These patients comprise merely a small proportion of all those treated at the Renal Clinic during the relevant period, only those who underwent bilateral renal vein and/or ureteric catheterization being included in the material.

Healthy volunteers were used as controls for determination of the PAH extraction by the right and the left kidney (see Table III). They consisted of 11 subjects (8 men and 3 women) aged from 18—27 years (average 20 years).

In certain cases, the basis of selection was that the diagnosis of chronic pyelonephritis was somewhat doubtful. In many patients who fulfilled several of the cardinal criteria of chronic pyelonephritis, the object was to ascertain whether or not involvement of the kidneys was asymmetric by means of a comparison between them with respect to such factors as the various parameters of function and the findings at roentgenologic examination. In addition, renal vein and ureteric catheterization were performed in a few patients with other renal diseases than chronic pyelonephritis to demonstrate whether involvement was symmetric or asymmetric.

For technical reasons, not all the patients

in the present series could be investigated regarding each of the eight cardinal criteria listed in Chapter III. The results of investigation nevertheless sufficed to classify them in groups by the clinical diagnosis, on the grounds described in the following. The results of the investigations on which this classification was based are given in Table IV. This table also shows how many patients in each of the groups were investigated with respect to all eight cardinal criteria and how many were investigated with respect to a limited number of them. The number of patients in whom the relevant number of criteria were fulfilled can also be inferred from Table IV.

Since my primary aim was to investigate the symmetry (asymmetry) of renal function, the patients were divided into a number of groups according to the diagnostic symptoms and signs described in Chapters III and IV.

— Cases in which it was considered doubtful whether symmetric or asymmetric renal function could be expected, *i.e.* chiefly chronic and acute non obstructive pyelonephritis (groups A1 and A2).

— Cases of renal disease in which it was evident that the two kidneys were not damaged to the same extent, *e.g.* chronic pyelonephritis with unilateral urinary tract obstruction such as a concretion or ureteric stricture as well as cases of *e.g.* arterial hypertension with stenosis of a renal artery, unilateral renal tuberculosis and unilateral hydronephrosis (groups B1, B2 and B3).

— Cases in which the damage could be expected to be of more homogeneous type and to affect both kidneys to about the same extent *i.e.* chronic glomerulonephritis the nephrotic syndrome essential hypertension and cases in which no evident renal damage was demonstrable (groups B4 and B5)

Classification of Patients

Group A1 Chronic non obstructive pyelonephritis fulfilling three or more cardinal criteria (denoted in the figures by *) 4 men and 63 women aged from 18—67 years (average 44 years)

Group A2 Suspected chronic non obstructive pyelonephritis fulfilling less than three cardinal criteria, as well as acute and recurrent acute non-obstructive pyelonephritis (◊) 2 men and 9 women aged from 20—63 years (average 39 years)

Group B1 Chronic pyelonephritis fulfilling three or more cardinal criteria and with signs of urinary tract obstruction (o) 1 man and 25 women aged from 16—61 years (average 43 years)

Group B2 Renal disease except pyelonephritis with unilateral or mainly asymmetric involvement (X) 4 men and 7 women aged from 17—62 years (average 42 years)

Group B3 Arterial hypertension with stenosis or aneurysm of the main renal artery (■) 5 men and 10 women aged from 30—69 years (average 54 years)

Group B4 Renal disease which could be expected to involve both kidneys more homogeneously and to about the same extent The nephrotic syndrome and chronic glomerulonephritis are denoted by ▲ renal papillary necrosis without signs of urinary tract infection by △ and essential hypertension by □ 7 men and 9 women aged from 14—53 years (average 35 years)

Group B5 Patients with symptoms from the urinary bladder and urethra, in whom involvement of the kidneys could not be ruled out (Λ) 5 women aged from 20—42 years (average 34 years)

For reasons of space all cases belonging to groups B1—B5 have been combined in the graphic representations I am fully aware that this category of cases is extremely heterogeneous

Group A1

This group contains cases of chronic pyelonephritis without demonstrable obstruction of the urinary tract It also includes cases of renal papillary necrosis with concomitant symptoms of chronic pyelonephritis If a renal papilla or other anatomic impediment to the urinary flow was demonstrated, the patient was assigned to group B1

Three patients had roentgenologic changes suggestive of unilateral renal dysplasia (cases 45 53 64) Such kidneys are regarded to be predisposed to pyelonephritis (75) Since no impediment to the urinary flow was demonstrable and the patients fulfilled the criteria for a diagnosis of chronic pyelonephritis they were included in this group The series contains only one patient with diabetes mellitus (case 30) She was assigned to group A1 since she fulfilled 7 of the 8 cardinal criteria Examination of a renal biopsy specimen disclosed slight glomerular lesions and fundus changes of the type seen in diabetics were found at ophthalmoscopy Her blood pressure was slightly raised and both her blood sugar and urinary sugar were maintained within normal limits by insulin therapy

Special mention can be made of certain patients who fulfilled only three of the diagnostic cardinal criteria In cases 35 and

CHAPTER V

CASE MATERIAL

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Healthy volunteers were used as controls for determination of the PAH extraction by the right and the left kidney (see Table III). They consisted of 11 subjects (8 men and 3 women) aged from 18—27 years (average 20 years).

In certain cases the basis of selection was that the diagnosis of chronic pyelonephritis was somewhat doubtful. In many patients who fulfilled several of the cardinal criteria of chronic pyelonephritis the object was to ascertain whether or not involvement of the kidneys was asymmetric, by means of a comparison between them with respect to such factors as the various parameters of function and the findings at roentgenologic examination. In addition, renal vein and ureteric catheterization were performed in a few patients with other renal diseases than chronic pyelonephritis to demonstrate whether involvement was symmetric or asymmetric.

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in the present series could be investigated regarding each of the eight cardinal criteria listed in Chapter III. The results of investigation nevertheless sufficed to classify them in groups by the clinical diagnosis, on the grounds described in the following. The results of the investigations on which this classification was based are given in Table IV. This table also shows how many patients in each of the groups were investigated with respect to all eight cardinal criteria and how many were investigated with respect to a limited number of them. The number of patients in whom the relevant number of criteria were fulfilled can also be inferred from Table IV.

Since my primary aim was to investigate the symmetry (asymmetry) of renal function the patients were divided into a number of groups according to the diagnostic symptoms and signs described in Chapters III and IV.

— Cases in which it was considered doubtful whether symmetric or asymmetric renal function could be expected, i.e. chiefly chronic and acute non-obstructive pyelonephritis (groups A1 and A2).

— Cases of renal disease in which it was evident that the two kidneys were not damaged to the same extent, e.g. chronic pyelonephritis with unilateral urinary tract obstruction, such as a concretion or ureteric stricture, as well as cases of e.g. arterial hypertension with stenosis of a renal artery, unilateral renal tuberculosis and unilateral hydronephrosis (groups B1, B2 and B3).

calcinoses. In this group as well hypertalcaemia may have reduced the renal concentration ability (*cf* group B1).

Group B3

All patients in this group had arterial hypertension. All but one had stenosis of the main renal artery, bilateral in one (case 126). The exception (case 117) had an aneurysm of both main renal arteries. Since operation was being considered, she was admitted to the Renal Clinic for bilateral investigation of renal function including Howard's test as a positive result of this test strengthens the indications for operation (*cf* Chap VI). She was re-evaluated twice at an interval of 8 months on the second occasion after operation of the aneurysm of the right main renal artery.

Six of the patients underwent operation for the renal artery stenosis but I did not make any detailed study of the results of operation regarding such factors as the anatomic conditions, pressure gradients and effect on arterial hypertension. When operation was performed, and verified the diagnosis, this is noted in Table IV.

As pointed out in Chapter VI, stenosis of a renal artery — due to *e.g.* an atheromatous plaque — may be present without accompanying hypertension (209).

None of the patients in this group fulfilled the minimum three cardinal criteria for a diagnosis of chronic pyelonephritis. It must however be pointed out that these patients were not as thoroughly tested in this respect as the others. As a rule they were tested for only 5 of the 8 cardinal criteria.

Group B4

I collected in this group patients in whom there was reason to presume that the renal

damage was homogeneous with little or no asymmetry between the kidneys. The diseases in question were renal papillary necrosis without signs of urinary tract infection or chronic pyelonephritis (2 cases), essential hypertension (4 cases), chronic glomerulonephritis (5 cases) and the nephrotic syndrome (5 cases). Although case 145 fulfilled three of the cardinal criteria of chronic pyelonephritis, a renal biopsy specimen showed slight glomerulonephritic lesions. It was not however possible to rule out unconditionally the existence of superimposed chronic pyelonephritis.

Group B5

The patients assigned to this group were investigated because chronic pyelonephritis was suspected. They all had urinary tract symptoms referred to the bladder and/or urethra and could not therefore be regarded as healthy, even though the renal function appeared to be largely normal and they did not fulfill the criteria of chronic pyelonephritis.

In a series reported by another author, cases in which an acute exacerbation had occurred in close association with the tests were excluded (10b). This may be justified when the degree of functional impairment is to be investigated. In the present study — in which the object was to compare the function of the two kidneys with respect to existing pathological processes — such cases were on the contrary regarded as highly suitable. Nor did I omit any patient on account of advanced age. It is true that renal function tests made in elderly persons often show decreased values (151, 264). However, my interest was not primarily focused on renal function in general but rather on the

39, the inflammatory process was of such long duration that there were no longer any signs of activity (*e.g.* rise in temperature pain in the flank, bacteriuria and positive sediment) In these cases the diagnosis was verified at autopsy Cases 36 and 48, in which only three criteria were fulfilled, comprised borderline cases between acute and chronic pyelonephritis It was difficult to decide whether it was a question of an acute, isolated attack of pyelonephritis or an exacerbation of a chronic course

Group A2

The patients assigned to this group were those with acute or recurrent acute pyelonephritis, and those suspected to have chronic pyelonephritis but who did not fulfil even the three criteria regarded as a minimum for this diagnosis Obviously, it cannot be ruled out that some of those considered to have acute pyelonephritis may have had renal damage of a chronic nature, but that any such functional defect was masked by the well maintained total function

Group B1

The patients in this group fulfilled at least three cardinal criteria of chronic pyelonephritis, but had some complicating disease of the urinary tract, *e.g.* urolithiasis, hydronephrosis or uretero vesical reflux

Case 103 had hyperparathyroidism with renal calculi This patient was investigated before parathyroidectomy (as was case 109 in group B2) Hyperactivity of the parathyroid hormone is known to produce an increase in both glomerular filtration rate and renal blood flow (98) In acute hypercalcaemia in animals injection of parathyroid hormone reduces the maximal renal concentration ability (73) and hypercalcaemia is associated with hyposthenuria (160)

This group also contains six patients with renal papillary necrosis (cases 84, 86, 92, 94, 95, 105) In these cases, the sloughed papillae formed an impediment to the urinary outflow

In case 91, chronic pyelonephritis was complicated by previously operated on perinephritis on the left side

None of the cases diagnosed as chronic obstructive pyelonephritis fulfilled less than three cardinal criteria

Group B2

The patients assigned to this group had renal disease which could be expected to be unilateral, or predominantly asymmetric even if both kidneys were affected Among them were cases of congenital hypoplasia of one kidney (cases 106-112), unilateral hydronephrosis (case 110), unilateral nephrolithiasis (case 111) and bilateral nephrolithiasis associated with hyperparathyroidism (case 109) None of the aforementioned patients fulfilled the three cardinal criteria regarded as a minimum for a diagnosis of chronic pyelonephritis

In the remaining cases three or more diagnostic cardinal criteria were fulfilled In two patients (cases 108 and 116) coincident chronic pyelonephritis and silent glomerulonephritis could not be ruled out (*cf* Chap III) Two patients had concomitant renal tuberculosis and chronic pyelonephritis (cases 113 and 114)

Finally one patient had suspected renal tubular acidosis (case 115) She had no haematologic changes characteristic of this disease nor a high urinary pH However pyelography disclosed intrarenal calcinosis denoted by the radiologist as typical of renal tubular acidosis In addition she had chronic pyelonephritis possibly secondary to the

in several of them, the investigations were made both before and during treatment. In most cases long term medication was initially confined to sulphamethizole (Lucosil® Lundbeck) or to a combination of sulphamethizole and sulphamethoxyypyridazine (Sulfapral® Astra) or in some cases to nitrofurantoin (Furadantin® Pharmacia) or penicillin (Cetacillin® Doktacillin® Astra). Periodically this medication was combined with antibiotic therapy after determination of the sensitivity of the invading organisms (42 44 45 104 183 184 186).

In the majority of cases treatment produc

ed marked improvement, both objective and subjective. A few patients became worse during the observation period with an associated deterioration in the results of the functional tests (*cf* Chap. X pp. 00—00). Consequently in some of the patients who underwent repeated tests the results varied greatly on the different occasions. In these cases I considered it most correct — as can be inferred from Table X — to record them in the plots so that each test occasion was represented by one value. This is why in most of the figures the number of values exceeds the number of patients.

functional difference between the two kidneys. It seems unlikely that the limitation of renal function which appears more or less consistently in elderly persons would affect one kidney more than the other. Possible exceptions are cases in which renal function is impaired on the grounds of local anatomic changes, *e.g.* enlargement of the prostate. Such cases have therefore been assigned to group B1.

There can scarcely be any doubt that the diagnosis was, in fact, correct in the cases classified as chronic non obstructive pyelonephritis (group A1), even if none of the cardinal criteria alone is specific to the disease. Moreover, it is improbable that these patients, all of whom were thoroughly investigated, had any other intercurrent diseases than those listed in Table IV.

In groups B1—B5 as well, the relevant diagnoses can be regarded as confirmed by the existence of characteristic symptoms and signs. As far as group A2 is concerned it consisted chiefly of patients who had acute attacks of pyelonephritis. Naturally, it cannot be ruled out that some of them actually had chronic pyelonephritis, but none fulfilled the minimum number of cardinal criteria required for a diagnosis of this disease. Consequently, although they have been combined with group A1, they have been given a special denotation (\diamond).

Thus in accounting for and discussing the results, the cases of chronic non obstructive pyelonephritis have been considered as a homogeneous group, and their values plotted in the same figures together with those in group A2. For comparison, the values in all the other groups (B1—B5) have been combined in corresponding figures. This implies that the latter plots also contain a number of cases of chronic pyelonephritis.

However, since all these cases were complicated by renal concretions sloughed renal papillae or other obstructions to the urinary flow, I considered it more correct to account for them together with the groups of other renal diseases. In view of this procedure the groups serving as comparative material with the cases of chronic pyelonephritis do, in fact, contain precisely a number of cases of this disease. This was nevertheless regarded as justified, because of the dominance of the complicating factors in these cases of secondary chronic pyelonephritis. They are easily distinguished in the plots since they are denoted by a special sign (\diamond).

The cases combined in the plots under the heading of other renal diseases form an extremely heterogeneous group in which such widely differing conditions as, *e.g.* the nephrotic syndrome and renal artery stenosis are represented. Consequently it must be emphasized that when this group is discussed, it is only as a group of patients with renal diseases other than chronic pyelonephritis, or those in whom pyelonephritis was not the salient feature. Obviously the combined group is not representative of any particular type of functional damage. On the other hand certain of the individual groups can provide an adequate basis for discussing the implications of the results from the diagnostic and pathophysiological aspects in the group in question. This applies especially to the well defined, thoroughly investigated cases of stenosis of the main renal artery (group B3). Such a discussion is however beyond the scope of the present study. For this reason only a brief account is given in the relevant connexions of the results in this group and in the other ones.

With respect to the patients with chronic pyelonephritis it must be pointed out that

consistency of the main renal artery to various split function tests Howard *et al* (106) and Connor *et al* (56) stated that in stenosis of the renal artery the urinary volume should be reduced by at least 50 per cent and the Na excretion by at least 15 per cent to motivate further investigation or exploration of the kidney. Both these criteria had to be fulfilled for Howard's test to be denoted as positive.

Hradkova & Schuck (107) calculated the tubular reabsorption of water in children by determining the concentration index of

Na $\frac{U_{Na}}{P_{Na}}$ (cf Chap II). No difference was

noted in this respect between a group with normal renal function and a group with anomalies of the urinary tract nor could any difference be demonstrated between the right and left kidney. However, most of the children in a third group consisting of cases of chronic pyelonephritis showed a difference between the kidneys as regards the reabsorption of water. These authors had no cases of arterial hypertension or stenosis of the main renal artery in their material.

It is difficult to avoid leakage of urine from the catheters and thus to make accurate quantitative measurements of the urinary volume. Rapoport (202) among others therefore suggested using the tubular rejection fraction in which he calculated the urinary excretion of endogenous Na and creatinine as well as the concentration of these substances in the blood but did not have to take the urinary volume into account. Schuck *et al* (224) used a formula to calculate the excretion of H_2O and Na (in relation to the filtered quantity) which did not necessitate determining the exact volume of urine per time unit. Birchall *et al* (21) also employed the relation between the

urinary concentration of Na and creatinine and found it to be better than clearance tests for confirming the diagnosis of unilateral obstruction of a renal artery.

Excretion of Hydrogen Ions and Ammonia

This has long been regarded as a true measure of tubular function (232-272). Since evaluation of the ability of the kidneys to acidify the urine necessitates loading with acidifying substances this method could be applied as a split function test in only a few of our cases.

The method of Rehn & Günzburg (205) consisting of loading the hydrogen ion excretion with HCl and $NaHCO_3$ respectively was not used in the present investigation. This method was further developed by Raabe (199) and Schneider (221). In my series the test was confined to loading with an acidifying salt (ammonium chloride). For further details see Chapter VII.

Several authors have suggested determining the NH_3 concentration in specimens obtained by ureteric catheterization (96-218, 220). The method has however a serious drawback, i.e. it cannot be used in unilateral or bilateral infection with urease forming bacteria — e.g. certain *Proteus* strains — since these raise the ammonia concentration. This implies that the method cannot be used as a generally reliable test.

Clearance Tests

A prerequisite for making clearance tests on specimens obtained by ureteric catheterization is that the urine can be collected quantitatively per time unit from each kidney. An account is given in Chapter VIII of the complications and sources of error associated with ureteric catheterization.

CHAPTER VI

CHOICE OF METHODS FOR DEMONSTRATING ASYMMETRY

It was important for the investigation to choose methods which provided information about the functional state of the kidneys and, at the same time, could be used with satisfactory reliability for each kidney separately. A brief survey is given in the following of the methods chosen. Naturally, the investigation could have been extended to include other functional tests and urine analyses of interest for evaluating the symmetry of renal function. One of the reasons for confining it to the tests described was that much more time and greater resources would otherwise have been needed. Moreover, I did not wish to include certain tests which might have influenced each other — An account is given in Chapter III of the methods used purely for diagnostic purposes, without a view to studying the symmetry.

Renal Concentration Ability

Determination of the osmolarity of the urine is definitely the most suitable way of measuring this parameter. The method was presumably applied for the first time to urine by von Koranyi (140) in 1897. The test is easy to perform and requires only an inappreciable quantity of urine. Many investigations have been reported which prove the value of ascertaining the concentration ability by determining the specific gravity or osmolarity of the urine obtained by ureteric catheterization as a measure of the degree of damage to each kidney (*cf* Table II).

Creatinine Excretion

The creatinine excretion has long been used as a gauge of renal function. Van Hoo genhuijze (105) seems to have been the first, in 1914, to determine the excretion in catheter specimens, after which the method was soon adopted by other workers *e.g.* Saheki (219), Ockerblad (178), 179), Lunoe (154), Yago (275) and Vuust (262) were among the first to describe determinations in catheter specimens of urine after administration of exogenous creatinine. With reservation for the errors inherent in the actual procedure of ureteric catheterization the method is well suited for investigation of each kidney separately. The sources of error of the method and its limitations are discussed in Chapter VIII.

Diuresis and Sodium Excretion

These functions are obviously also appropriate for bilateral functional analyses here as well with reservation for the sources of error due to the catheterization procedure.

Experimental polyuria has been tried (245) based on the observation that water loading results in polyuria when a kidney is healthy whereas diuresis is smaller when a kidney is diseased. The method is however time consuming and the effort strenuous for the patient nor is it reliably undetrimental. Since increasing interest has been focused in recent years on diagnosing stenosis of renal arteries extensive work has been devoted to correlating the existence of

Among the methods that were not used in the present series mention can be made of a few which were earlier applied as separate functional tests.

Urea Concentration

Determination of the urea concentration in urine was much used for many years as a test of the function of each kidney. The method was introduced by Rosing (215) in 1892. Urea was administered intravenously (86, 138, 275) or by mouth as in McLean's test (111, 166, 201, 242). Gothgen (86) who worked with urea clearance tests for several years stated that the value of McLean's test as a measure of unilateral renal function could not be proved. He observed — as did Husfeldt & Aikjaer (111) — several cases in which the diseased kidney showed as good function as the healthy one or better. This was ascribed partly to the diuretic effect of urea.

Dye Tests

Dye tests with e.g. methylene blue, indigo carmine or phenolsulphonphthalein (phenol red) have long been the most extensively used tests of bilateral renal function. The substances can be injected i.v. The method was originally introduced in 1892 by Kutner (114) as chromocystoscopy. It implied that after injection of e.g. indigo carmine the excretion of the dye was studied by inspection of the ureteric orifices through the cystoscope. It was sometimes difficult to judge the intensity of colour (260). Better results were obtained when ureteric catheters

were passed. Hanzawa (90) compared the refractive specific gravity, decrease in freezing point and urea concentration in urine and found the indigo carmine test to be the most accurate. Hellström (95) and Chwalla (54) were more critical of this test. They found that the results based on a large series of cases did not always permit conclusions on the degree and extent of the anatomic processes in one or both kidneys.

Approximately the same results as with the indigo carmine test were obtained in determinations of the phenolsulphonphthalein excretion (46, 217, 249).

Although the excretion of a dye as a bilateral test of renal function has lost ground in the past few decades it is still used (161).

Radiorenography

This method which was devised by Winter (270) is obviously of great importance for estimating the symmetry of renal function. The literature is already voluminous (158).

The *electrorenogram* which is a recording of the electric potentials from the renal parenchyma is also used to demonstrate differences between the function of the two kidneys (136).

Arterio-venous haemoglobin and oxygen tests. Arterio-venous haemoglobin and oxygen differences have been compared with EPH_{H} simultaneously in both kidneys in healthy subjects and in patients with various kinds of renal disease (181).

The creatinine clearance test with administration of exogenous creatinine was introduced in 1926 by Rehberg (203) Molle *et al* (170) were the first, in 1929, to use the urea clearance as a test of renal function. As early as 1930, Lunoe (154) calculated the creatinine clearance (after loading) and urea clearance in the urine of each kidney. Many studies have subsequently been published, based on separate clearance determinations in various renal diseases. The creatinine and inulin clearances have been used, as well as the PAH clearance (19, 20, 21, 27, 50, 51, 53, 94, 102, 108, 137, 139, 142, 145, 162, 173, 175, 187, 189, 214, 222, 228, 261).

In recent years the improved possibilities of diagnosing stenosis of renal arteries have in fact increased the importance of separate clearance determinations (5, 6, 87, 134, 156, 157, 192, 226, 233, 238, 240, 259).

Despite the sources of error inherent in separate clearance determinations they were performed in a number of cases in my series chiefly in connexion with renal vein catheterization.

Renal Extraction of PAH and Diodrast

As far as earlier studies of these determinations are concerned, reference is made to Chapter VII (Renal vein catheterization).

Roentgenologic Examination

Naturally this method of examination — especially *excretory pyelography* — has been used to evaluate the state of each kidney ever since it became possible with the help of roentgen rays to visualize the contours of the kidney with the rest of the urinary tract and the renal parenchyma. There is

a wealth of literature on the subject, most of which is beyond the scope of the present study. Pertinent references are given in Olsson's paper (182).

Mention can, however, be made of one method used for diagnosing stenosis of a renal artery. Butchall *et al* (21) suggested hydrating or dehydrating the patient in connexion with pyelography. The method was further elaborated by Amplatz (1) and Brannan *et al* (26). In patients with renal artery stenosis the nephrogram effect in the stenosed kidney appears later and persists for a longer time when the patient is dehydrated. Pyelography has been regarded by most authors as inferior to quantitative physiologic methods for judging renal function (52, 66, 67, 274).

Aortography and *renal angiography* are also methods of importance in studies of symmetry. They disclose the anatomic details in the form of narrowings of the lumen, their extent and degree as well as the existence of any post stenotic dilatation. Several authors have however pointed out that renal artery stenosis (209) of varying degree and multiple renal arteries (61) are not infrequently demonstrated without the patient having controllable renal hypertension or a rise in blood pressure of any kind.

In my cases renal angiography was some times of value in the diagnosis of chronic pyelonephritis as well. This applied particularly to such questions as the extent of pyelonephritic scarring, size of the kidney and as a differential diagnostic aid. *Abstinention cystography* e.g. when vesico renal reflux was suspected and *retrograde pyelography* were also found to be valuable complements to other roentgenologic examinations.

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Table VII *No mal values for some tests of renal function*
The relevant values are according to reports from the Clinical Central Laboratory
St Erks Sjukhus and experience at the Renal Clinic

Seum creatinine	0.9-1.4 mg/100 ml
Renal concentration ability	> 800 mOsmol/lit
Renal acidifying ability	< pH 5.3
Excretion of radioactive Diodrast (whole blood)	49-86% (16)
Excretion of PAH	See Table III
Renal plasma flow (determined with PAH)	650 ± 31.9 s 150 ml/min 1.73 m ² BSA (14)
PAH clearance	589 ± 29.3 s ± 138 ml/min 1.73 m ² BSA (14)
Endogenous creatinine clearance	32 healthy volunteers (18 women and 14 men aged 16-67 years)
Women (mean)	89.9 ± 6.7 l s ± 28.49 ml/min 1.73 m ² BSA
Men (mean)	96.0 ± 8.06 s 30.16 ml/min 1.73 m ² BSA
Inulin clearance	119-4.6 s ± 14.4 ml/min 1.73 m ² BSA (14)

the concentrations used in arterial blood PAH in urine was determined by the same method but without precipitation of protein.

No PAH determinations were made in patients who during the previous 48 hours had taken any drugs that might have disturbed the investigation (e.g. sulphonamides, PAS or Palerol comp®). It has been shown that an acetylation of PAH in the kidneys does not occur as of negligible influence on the determination of PAH (176). My series contains only one patient with diabetes mellitus and she had no glycosuria at the time of investigation. This is worth mentioning since glycosuria may cause errors in the PAH determination (72).

Radioactive Diodrast was determined by gamma counting of haemolyzed blood at the photopeak of ¹³¹I 0.38 MeV as described by Bergstrom *et al* (16).

Methods of Clinical Investigation

Renal excretion

Cournand & Ranges (60) elaborated the method of catheterizing the heart introduced by Tönnmann (79) to include other parts of the circulatory apparatus. It was not until Warren *et al* (265) described a method for

obtaining blood from the renal veins in man with the help of a Cournand catheter that it became possible to determine the renal excretion of such substances that are excreted in large quantities in the urine e.g. PAH and Diodrast. When the PAH excretion could be determined at renal vein catheterization this value and the renal PAH clearance could be used to calculate the renal plasma flow with much greater accuracy than before. The method described by Warren *et al* (265) implies a great advance in our possibilities of studying the physiology and pathophysiology of the kidneys in man. The method is however associated with certain difficulties and risks.

With the technique now used which was introduced by Seldinger (227) the catheters are advanced through a femoral vein. This has considerable advantages. In view of several other technical improvements the method is now so simple and has such slight risks that it has become one of the routine methods at our clinic.

Except in healthy experimental subjects renal vein catheterization was preceded by excretory pyelography to evaluate such factors as the size of the kidney and position of the renal hilus. In addition all patients were investigated for the existence of any

CHAPTER VII

METHODS

The chemical analyses were performed at the Central Laboratory of the hospital, whose staff has great experience of making such determinations. Some normal values for renal function are given in Table VIII.

Analytic Methods

Osmolality of urine The depression of the freezing point was determined with a thermometer and Wheatstone bridge, using a modification of the apparatus described by Bowman *et al* (23).

Creatinine Creatinine in serum was determined in the Folin Wu filtrate according to Haugen's modification (93) of Brod & Sirota's method (32). Creatinine in urine was determined directly with picric acid. The method is not specific, since it does not take into account the influence of chromogens.

Sodium and potassium in serum and urine were determined in an Eppendorf flame photometer (17).

Chlorides in serum and urine were determined by Brun's modification (35) of the method of Shales & Shales.

Serum bicarbonate (alkali reserve) was determined by the titrimetric method of Van Slyke *et al* (231) using a Beckman automatic potentiometric titrator.

Protein in urine This was determined by the biuret method after precipitation with trichloroacetic acid and redissolving the precipitate in alkaline biuret reagent.

pH of urine The determination was made

electrometrically (Radiometer's pH meter). This was done as soon as possible after the urine was voided to obviate any change due to formation of ammonia and loss of CO_2 .

Ammonia and titratable acid These determinations were made in a large number of cases on bladder and ureteric urine, but since it was subsequently found that this had not been done consistently, the results have not been included. Moreover, the urine was sometimes strongly infected by urease-forming bacteria, and determinations of the ammonia content were then regarded to be of limited interest.

Inulin was initially determined by Josephson & Godin's modification (120) of Corcoran & Page's method (58) using a diphenylamine reagent. Later the method of Heyrovsky (97) was used instead.

PAH The technique for determining PAH was changed in the course of the investigation. Initially, the method of Smith *et al* (235) was used, but was later replaced by that of Brun (36a). The two methods give compatible values. When the protein was precipitated for determining the PAH in arterial blood, the plasma dilution was 1:15. When determining the low concentrations of PAH in renal vein blood, the dilution was 1:10. In precipitation of protein a lower dilution cannot be used since the PAH may then be lost with the precipitate. At low concentrations in renal vein blood the values were read against a blank using the method described by Smith *et al* (235). The blank is negligible in determination of

the brachial vein. The infusion solution consisted of 450 ml of physiologic saline with the addition of 20–40 ml of 20 per cent PAH. (In a few patients with signs of greatly impaired renal function only 5–10 ml of PAH were given.) Fifty ml of a 10 per cent inulin solution were added to the infusion solution of which 20–30 ml were given as a priming dose followed immediately by continuous infusion at a rate of 2 ml/minute. A pump designed by Ek (71) was used for this purpose. These measures allowed the concentration of PAH in plasma to be kept on a fairly constant level of 1–4 mg/100 ml, and that of inulin at about 50 mg/100 ml.

To check that the catheter in the renal vein was in the proper position during catheterization a rapid approximate method was used to determine the PAH concentration in heparinized whole blood from the renal vein and arterial blood. When the preliminary analyses showed no definite difference in concentration it was considered probable that the catheter tip did not lie in the renal vein; its position was then checked and adjusted if necessary. As a rule 5 to 10 determinations were made on blood from one or both kidneys at intervals of 5–15 minutes.

In some cases a solution containing 0.15 ml/kg body weight of a 20 per cent solution of the sodium salt of PAH mixed in the syringe with 0.05 ml/kg body weight of 2 per cent Xylocaine epinephrine® was administered 1/2 to 1 hour before the examination. The solution was given as a slow intramuscular injection in the superior outer quadrant of the buttock as described by Buche (40). A smaller quantity of PAH was given to patients with impaired renal function.

To permit samples to be taken of the peripheral blood a polythene catheter was inserted in the brachial artery. The infusion was given through a plastic tube introduced into a cubital vein without local anaesthesia whereas arterial puncture was preceded by local anaesthesia with 2 per cent Xylocaine®.

Titration of the depression limit for renal extraction of PAH. In all essentials the method used was that described by Bergstrom *et al* (16). Briefly it implies that a catheter was passed into a renal vein and that repeated determinations of the renal extraction of PAH were made while the PAH concentration in arterial blood was increased stepwise. As a rule it was tried to start at 1–3 mg/100 ml serum, after which the determinations were repeated at about 5, 10–15 and 15–20 mg/100 ml. Little regard was paid to PAH clearance since clearance determinations are of slight or no value with a varying high PAH concentration in plasma. In some cases bilateral determinations were made simultaneously.

Josephson *et al* (121–123) found that Diodrast and PAH competed with each other with respect to tubular excretion as has been shown to apply to other pairs of substances (232). They presumed that Diodrast and PAH are excreted by means of a common enzyme system or at any rate by an enzyme system in which some step must be in common. Consequently it could be expected that the extraction of Diodrast would also start to rise when the PAH extraction was increased so that its depression limit was reached. In the experiments 0.10–0.25 $\mu\text{Ci}/\text{min}$ (totally 25–35 μCi) of carrier free radioactive Diodrast (Abbot) was given in the same infusion solution as inulin and PAH. Since the extraction was calculated from the

cardiovascular disease, by means of physical and roentgenologic examination of the heart, diurnal recording of the blood pressure, and ECG recording. Since renal vein catheterization places little strain on the patient and is done under local anaesthesia poor general condition was considered to be the only contraindication.

Initially renal vein catheterization and ureteric catheterization were combined (39 investigations), but this procedure was subsequently abandoned, as being too troublesome for the patient. The combined examination was, however, made in patients with arterial hypertension, when this was suspected to be of renal origin. In these cases, fluids were not restricted. In the other cases, the bladder urine was collected through a catheter in the bladder at renal vein catheterization (50 investigations), and ureteric catheterization was performed as a separate examination (116 investigations). The technique of renal vein catheterization has been described in detail by Edvall (70), but a brief account is given in the following since certain modifications were made in the present study.

As in Edvall's technique, the catheter was introduced through the femoral vein. The site of puncture in the inguinal region (usually on the right side) was infiltrated with 20—30 ml of 0.5 per cent Xylocaine® after palpating the femoral artery. The needle used for puncture of the femoral vein was the curved cannula with mandrin designed by Gidlund (83). When the needle had been advanced for a short distance towards and through the wall of the vein the mandrin was removed and the patient instructed to bear down. When venous blood was expelled under pressure a metal guide of Seldinger's model (227)

was introduced. All manipulations of the guide and catheter were made with great caution, since incautious contact with the vessel walls may elicit a vasovagal reaction with a resulting fall in blood pressure.

When the metal guide had been advanced for a few decimetres the puncture needle was withdrawn and a catheter introduced over the guide. This catheter was of radiopaque material (180) and curved before hand (83), with a diameter of curvature of 3—4 cm. It was advanced, under fluoroscopic control beyond the site of origin of the renal vein after which the guide was withdrawn. The catheter was then passed slowly downwards by moving the tip, and usually slid easily into the renal vein. In some cases a catheter was inserted into each of the renal veins to obtain simultaneous samples. Both catheters were introduced through the same puncture hole.

The movements and position of the catheter (or catheters) were checked continuously on a television screen, which reproduced the roentgenologic picture of the current position of the catheter. Initially a Philips Image Amplifier was used for fluoroscopy during catheterization but this required the examination to be made in almost complete darkness and in an uncomfortable position for the examiner. Consequently it was a great advantage when the picture could be reproduced on a closed circuit TV screen (Philips TTV chain) placed near the patient's head. This implied that the image was clearer and the examination could be carried out in subdued room illumination. Moreover this method requires a much lower intensity of roentgen irradiation.

In most cases PAH was administered as an i.v. infusion through a plastic catheter in

Initially ureteric catheterization was combined with renal vein catheterization (cf p 50) In the majority of cases however it was done at a separate session

Premedication consisted of 20 mg of 1 N butylscopolammonium bromide (Buscopan®) or 80 mg of papaverine hydrochloride injected i.m. Sometimes a short acting barbiturate (5 ethyl 5 isoamylbarbituric acid Pentymal®) in a dose of 0.1 g was given as well No drugs were administered while the examination was being made

After local anaesthesia of the urethra with Xylocaine gel® a Brown Buerger cystoscope (24 Fr) was introduced with great caution The ureteric catheters were of 5—7 Fr calibre and had lateral holes about 1 cm apart arranged spirally in the first 10 cm from the tip (154) If possible the catheters on both sides were of the same calibre They were advanced 12—15 cm in the ureters The cystoscope was then withdrawn, and a bag catheter (Foley 16—18 Fr) was passed into the bladder to check that no urine leaked along the sides of the ureteric catheters In some cases a check was made of possible leakage by means of chromo-cystoscopy and inspection of the ureteric orifices as recommended by e.g. Rothauge (214) and Vondra (261) I injected a dye i.v. and then observed through the cystoscope left in the bladder whether any coloured urine escaped from the ureteric catheters I did not use suction as suggested by Lunöe (154) in view of the risk of the catheter adhering to the wall of the ureter (102)

Collection of urine When the catheters were in position the bladder was thoroughly emptied by means of irrigation with physiologic saline followed by insufflation of air This was done to enable any leakage to be checked as well as to prevent the renal

haemodynamics and function from being influenced by distension of the bladder by urine (193) The first few millilitres of urine from the ureters were discarded since it could not be ruled out that they contained bladder irrigation fluid Actually I waited for 15—20 minutes before starting to collect specimens for analysis since the first portions of urine are regarded to be more affected by the manipulations than are later portions (cf Chap VIII) The mouths of the ureteric catheters were placed as low as possible to take advantage of the siphon effect

The urine was collected simultaneously from each kidney in graded tubes which were changed concurrently and the time carefully noted Quantities ranging from 1 to 8 ml were required for the analyses Several specimens were collected from each ureter and the mean value of determinations of e.g. osmolality electrolyte content and clearance was taken as the basis for evaluating the function

The urinary bladder was carefully emptied after each period The first specimens from each ureter were taken under sterile conditions for bacteriologic examination as was urine from the bladder in connexion with cystoscopy Before the catheters were withdrawn the patient was usually given a spasmolytic and/or analgesic agent

Complications The results were discarded, and the examination was sometimes repeated, if any of the following events occurred

— The urinary flow was so small that a sufficient quantity could not be collected for the analyses

— The urine contained an appreciable amount of blood

— There was poor agreement between the results in the different periods

number of counts in blood from an artery and the renal vein, it expresses the extraction in whole blood and not in plasma, as in the case of PAH

In the present investigation I used the method of Josephson *et al* (121, 122) as modified by Bergstrom *et al* (16). On technical grounds, the renal extraction of radioactive Diodrast was determined in only five cases, in one of them bilaterally (Fig 16). This case is given as an illustration of an attempt to check the result in studies with varying PAH extraction

Complications In my series, complications which necessitated discontinuing the examination occurred in only two cases. Thus, in the 131 renal vein catheterizations in which bilateral determinations were made (89 catheterizations), a haematoma in the groin with a transient fall in blood pressure occurred in one patient, and another also had a temporary fall in blood pressure, probably caused by vasovagal reflex (case 2). The former patient was excluded from the material, whereas the results of tests in the latter patient were included up to the time when the first reaction was observed

To decrease the risk of haematoma, slight pressure was applied to the site of puncture during the examination. When the catheter had been withdrawn, pressure was applied for a few hours by means of sandbags. No signs of thrombosis of the punctured vessel nor of embolism were observed (152)

Ureteric catheterization

Interest in studying the function of one kidney in relation to that of the other has existed for a long time. The work of Nitze & Casper (145), performed around 1890 led to ureteric catheterization being used

more often than before as a method of examination. As early as about 1902, Nitze constructed a ureter occluding catheter with a rubber balloon which surrounded the catheter and was filled with water after the catheter had been passed (145). The construction was, however, so clumsy that it was difficult to use. Attempts have also been made to divide the bladder into two halves by a so called vesical separator, but this technique is best suited to animal experiments (27, 145)

Others have succeeded in occluding one ureter by external pressure (19), and collecting urine from the other ureter by passing a catheter into the bladder. Some authors (65, 131, 149-214) have passed only one ureteric catheter, the urine from the other ureter being collected in the bladder, and led off through a cystoscope or vesical catheter (51, 55, 145, 149-187, 202, 233)

A somewhat varying technique has been used for ureteric catheterization. As a rule the catheters have been of relatively large calibre (5-9 Fr), and often provided with lateral holes in the tip. They have been advanced into the ureter for 15-25 cm. Ureteric catheters with lateral holes were introduced by Lunoe (154). He made a spiral pattern of 10-12 lateral holes about 1 cm apart in the first 10 cm of the catheter

In the present series, the procedure was as follows. All chemotherapeutic or antibiotic therapy was discontinued when the patient was hospitalized for investigation. Renal vein and ureteric catheterization as well as the other examinations were performed as soon as possible. Treatment of any urinary tract infection on the basis of the antibiogram, was postponed until immediately after catheterization

Initially ureteric catheterization was combined with renal vein catheterization (cf p 50) In the majority of cases however it was done in a separate session

Premedication consisted of 20 mg of 1 N butylscopolammonium bromide (Buscopan®) or 80 mg of papaverine hydrochloride injected i.m. Sometimes a short acting barbiturate (5 ethyl 5 isopropylbarbituric acid Pentymal®) in a dose of 0.1 g was given as well No drugs were administered while the examination was being made

After local anaesthesia of the urethra with Xylocaine gel® a Brown Bueger cystoscope (24 Fr) was introduced with great caution The ureteric catheters were of 5—7 Fr calibre and had lateral holes about 1 cm apart arranged spirally in the first 10 cm from the tip (154) If possible the catheters on both sides were of the same calibre They were advanced 12—15 cm in the ureters The cystoscope was then withdrawn and a bag catheter (Foley 16—18 Fr) was passed into the bladder to check that no urine leaked along the sides of the ureteric catheters In some cases a check was made of possible leakage by means of chromo-cystoscopy and inspection of the ureteric orifices as recommended by e.g. Rothauge (214) and Vondra (261) I injected a dye i.v. and then observed through the cystoscope left in the bladder whether any coloured urine escaped from the ureteric catheters I did not use suction as suggested by Lundö (154) in view of the risk of the catheter adhering to the wall of the ureter (102)

Collection of urine When the catheters were in position the bladder was thoroughly emptied by means of irrigation with physiologic saline followed by insufflation of air This was done to enable any leakage to be checked as well as to prevent the renal

haemodynamics and function from being influenced by distension of the bladder by urine (193) The first few millilitres of urine from the ureters were discarded since it could not be ruled out that they contained bladder irrigation fluid Actually I waited for 15—20 minutes before starting to collect specimens for analysis since the first portions of urine are regarded to be more affected by the manipulations than are later portions (cf Chap VIII) The mouths of the ureteric catheters were placed as low as possible to take advantage of the siphon effect

The urine was collected simultaneously from each kidney in graded tubes which were changed concurrently and the time carefully noted Quantities ranging from 1 to 8 ml were required for the analyses Several specimens were collected from each ureter and the mean value of determinations of e.g. osmolality electrolyte content and clearance was taken as the basis for evaluating the function

The urinary bladder was carefully emptied after each period The first specimens from each ureter were taken under sterile conditions for bacteriologic examination as was urine from the bladder in connexion with cystoscopy Before the catheters were withdrawn the patient was usually given a spasmolytic and/or analgesic agent

Complications The results were discarded and the examination was sometimes repeated if any of the following events occurred

— The urinary flow was so small that a sufficient quantity could not be collected for the analyses

— The urine contained an appreciable amount of blood

— There was poor agreement between the results in the different periods

— The catheter became occluded, and patency could not be restored by any of the following measures

To restore passage in the catheter when the flow was arrested, 1—2 ml of air were insufflated. Neither distilled water nor physiologic saline was used, since this would have disturbed the determinations. Some times a blood clot was expelled, after which the flow started again. In some cases the catheter was exchanged for another one. If patency was not restored despite these measures the ureteric catheter was withdrawn and the urine from the relevant side was collected in the bladder, and allowed to flow out through the vesical catheter. In a few cases the examination was discontinued.

Since the complications associated with ureteric catheterization are well known, they need not be discussed further. It must, however, be mentioned that such complications arose on a few occasions in the present material. For example bilateral occlusion of the ureteric catheters occurred in one case, due to the presence of blood clots, with resulting oliguria which was subsequently relieved. This case is not included in the results.

Many patients experienced transient pain during catheterization and sometimes after it as well. These cases have not been excluded. Nor were any cases excluded on the grounds of mild bleeding. Nevertheless, it cannot be ruled out that the inappreciable complications observed in some patients in the form of pain and sometimes a slight fall in blood pressure may have influenced the results (cf Chap VIII).

I found no signs of cystoscopy or ureteric catheterization having elicited an ascending urinary tract infection in any case. This is in agreement with the experience of several

authors (8 113 216 253), although others maintain that there is a certain risk of this complication (155 171, 204, 230).

No prophylactic antibacterial treatment was given, but therapy was generally instituted immediately after this examination, on the basis of antibiograms made on specimens of urine taken shortly before it.

Endogenous creatinine clearance

This was determined as the apparent creatinine clearance, and was generally done in conjunction with determination of the PAH clearance. This applied both to the 2 periodic determination and to that made in connexion with renal vein catheterization. In a few cases the 24 hour clearance of creatinine was determined.

Inulin clearance

This was determined only in the patients who underwent renal vein catheterization. The inulin was administered in the same infusion solution as PAH. The quantity given was calculated to achieve a plasma concentration of about 50 mg/100 ml. Initially, a Czech inulin was used, later replaced by a German one and recently, by an American preparation.

PAH clearance renal plasma flow and PAH extraction

PAH clearance : As already mentioned this determination was often combined with that of endogenous creatinine clearance. At 6 a.m. the patient drank 2 glasses of water and 1 hour later the same quantity. Fasting was not required but smoking and drinking of tea or coffee were not allowed. If the patient was being treated with sulphonamides PAS or tropenizilium bromide piperylon maleate (Palerol®) this medication was discontinued for a few days before the test.

The test was started at about 8 a.m. when the patient was given PAH in a dose related to the body weight as an intramuscular injection together with Xylocaine norepinephrine[®] (10). The clearance determination was started about 1 hour later. As a rule two consecutive periods of about 60 minutes each were determined. After each period the bladder was emptied as completely as possible and a venous sample was taken. The patient was not catheterized unless she had difficulty in emptying her bladder or was menstruating. In a few patients — chiefly men — the duplicate determinations showed a lack of agreement. For this reason the values for PAH and endogenous creatinine clearance are listed only when the difference between the periods was less than 25 per cent of the higher value.

The PAH clearance was sometimes determined in connexion with renal vein catheterization with both ureteric catheters in place. The patient was then deprived of fluids for 24 hours before the test since ureteric catheterization was combined with the test of renal concentration ability. In these cases PAH was given together with inulin as an iv infusion at a constant rate as previously described. In these cases the renal vein blood was analyzed in a way that allowed the extraction of PAH to be determined. It was therefore regarded as unimportant if this concentration exceeded the diuresis limit in patients with impaired renal function. Obviously if this occurred the clearance value was worthless.

Renal plasma flow. This was determined in the same way as the PAH clearance but by analysis of renal vein plasma.

Renal PAH extraction. This was calculated from the concentration of PAH in arterial and venous blood.

Renal extraction of radioactive Diatrizoate

This was determined by the method of Josephson *et al* (121, 122) as modified by Bergström *et al* (16) cf pp 51—52.

Renal concentration ability

The test was made after the patient had been given a dry diet and deprived of fluids for 24 hours. Vasopressin (Pitressin tannate[®] in oil) in a dose of 5 I.U. was injected i.m. at 8 p.m. in the evening before the test. The osmolarity was determined in the urine voided at 8 and 10 a.m. on the following morning. The patient was prepared in the same way when the test was made with catheters passed into the ureters to determine the urinary concentration ability of each kidney separately.

Water loading test of Volhard

No fluids were allowed after 8 p.m. in the evening preceding the test. At 8 a.m. on the following morning the patient urinated, was weighed and then drank 1 litre of water as quickly as possible without discomfort. The patient then emptied his bladder at intervals of 1 hour for the next 4 hours after which he was weighed again. The quantity of urine was measured and the osmolarity determined as soon as was feasible. In no case did the water load cause nausea or other signs of water intoxication.

Renal acidifying ability

This test was made only in cases in which there was considered to be no risk of metabolic acidosis. The patient was given ammonium chloride in a dose of 0.1 g/kg of body weight per day on 3 consecutive days. The daily quantity was divided into three doses given in the form of tablets. The pH and content of ammonia and titratable acid were

determined in the first urine specimen on each day. The last specimen tested was the 4th day's morning urine. The specimens were preserved with one drop of toluene. When pH, ammonia excretion and titratable acid were determined in catheter specimens from the ureters, the test was sometimes preceded by acidification as just described. When this was done, it is noted in the tables.

Renal biopsy

This was performed with a modified Vim Silvermann needle using the technique of Kark & Muehrcke (126). The specimen was transferred to formalin, and sent to the Department of Pathology for examination.

Urinary sediment

In some cases, the technique suggested by Sternheimer & Malbin (243) was used to stain the leukocytes, with their characteristic, large vacuolated cells, which are pale, bluish in colour and of varying shape. In addition, a semi quantitative determination was made of the cells in a given volume of sediment from the 24 hour urinary output. The microscopic evaluation of the sediment in all cases of pyelonephritis was made by the same experienced examiner, before the patient had been given any therapy or had undergone any investigations.

Urine culture

This was done in all patients and a quantitative bacterial count was made in most of them. In men a mid stream specimen was collected, after washing directly in a sterile container. Since the end of 1959 women patients have, with few exceptions emptied their bladder through a sterile glass tube 120 mm long and 12 mm in diameter (45) held against the urethral orifice by the patient or an attendant. A mid stream specimen

was then taken. A specimen collected with a tube held against the urethral orifice like a vesical catheter specimen, raises the bacterial count only inappreciably, whereas in women an ordinary mid stream specimen often gives bacterial growth, partly due to contamination from the external genitalia or urethra. In the few cases in which the glass tube method was impracticable, a mid stream specimen was taken after thorough washing, being careful to avoid the urinary stream coming into contact with the labia majora.

In quantitative counts of the bacteria serial tenfold dilution plate counts were used whereas in non quantitative culture the urine was undiluted. The borderline for significant bacteriuria ($> 100,000$ bacteria/ml urine) applies only if the patient is not being treated with chemotherapeutics or antibiotics. A number of additional factors must be taken into account when evaluating the results of culture, e.g. pH and dilution of the urine, ureteric obstruction — which may give a false negative result — and time of taking the specimen (first morning specimen or random specimen). To decrease the sources of error in the present investigation, the procedure for taking the specimens was standardized, and they were transported directly in the morning to the laboratory where they were dealt with at once.

In almost all the patients, culture of morning urine on Lowenstein medium and inoculation of guinea pigs for detection of tubercle bacilli were performed on 3 consecutive days.

Blood pressure determinations and examination of ocular fundi

A 24 hour curve for the arterial blood pressure was generally recorded on the 1st

or 2nd day of hospitalization. The measurements were made at approximately 2 hour intervals with the patient confined to bed. Unless otherwise noted in the tables all hypotensive therapy had been discontinued for at least 5 days before the recoding.

The ocular fundi were inspected the stages of retinopathy (I-IV) being defined according to Keith & Wagener.

Roentgenologic Examination

All these examinations were carried out in the Department of Roentgenology of the hospital under the supervision of experienced roentgenologists.

In *excretory pyelography* the contrast media used were three iodo compounds (Hypaque® Winthrop or Urografin® Schering). *Retrograde pyelography* and/or *urethrocytography* were performed in a few cases and one of the aforementioned contrast media was generally used. The ureteric catheter for retrograde pyelography was of 5-7 Fr calibre and had only one or two holes in the tip. It was advanced into the renal pelvis. In some cases serial radiograms with a film changer (83) were made during slow withdrawal of the catheter and concurrent injection of contrast medium. In this way it was sometimes possible to visualize the changes in the renal pelvis or ureter responsible for poor opacification of the kidney at excretory pyelography.

Selective renal angiography was performed by the method of Seldinger (227) using an Odman catheter (180) bent according to Gidlund (83).

Calculation of

Results and Statistical Methods

Conventional statistical methods were used.

When evidently erroneous results of extraction determinations had been excluded (cf p 63) the mean was recalculated from the remaining values. As far as the renal extraction of PAH is concerned this applied to 5 determinations. In these cases certain of the erroneous values are probably to be ascribed to the renal vein blood being mixed with blood from the vena cava. It cannot however be completely ruled out — even if such an explanation seems highly unlikely — that a lower value for PAH extraction might have been due to the renal vein blood coming from a part of the kidney where the PAH extraction was lower in view of more extensive parenchymal damage. If this was actually the case it implies that the procedure of exclusion introduced an error in evaluation of the results.

When calculating the mean of clearance and RPF determinations with more than one period the values for each period were weighted against each other taking the time factor into account. When the total clearance was calculated for both kidneys these values were correlated to a body surface area of 1.73 m². This conversion was not however done when a comparison was made between the clearance by the right and the left kidney since the percentage difference between them was calculated.

When a single determination was made of the osmolality sodium, potassium and creatinine concentration in the urine from one kidney only the value for the corresponding period on the other side was included.

Recording of symmetry and asymmetry

When estimating the way in which renal disease had affected the symmetry of renal function I started out by comparing the size of the relevant parameters on either side.

Such a comparison is however, apt to be misleading. This is because a difference which appears inappreciable when the kidneys are functioning normally may, in fact, represent a great discrepancy in function in severely damaged kidneys, where *e.g.* extraction of PAH, and excretion of water, electrolytes, PAH and creatinine may be greatly decreased. Consequently, when accounting for the results, I have given most of the differences — not in absolute figures — but as the percentage difference between the kidneys as a measure of the asymmetry. The difference was calculated as a percentage of the function of the better kidney, measured

by the same parameter, and not as a percentage of the mean value, as described in Chapter I.

It would perhaps have been more correct to base the calculation on the functional impairment in the poorer kidney, and thus to have obtained a more accurate measure of how far the disease had advanced. However, when the difference is calculated as a percentage of the function of the better kidney, the range will naturally be smaller than if the corresponding calculation had been made on the poorer kidney, since the maximum difference cannot exceed 100 per cent.

SOURCES OF ERROR OF THE CLINICAL PHYSIOLOGIC METHODS

General Physiologic and Analytic Aspects

When a comparison is to be made between the two kidneys particular regard must naturally be paid to the reliability of the methods used. Actually the battery of renal function tests is relatively limited and only certain details of renal function are amenable to testing. Furthermore none of the tests that can be applied in an investigation of this type is selective for a given function even if some of the tests are influenced chiefly by an isolated partial function.

The fact that so many methods have been used to investigate patients with renal disease indicates that none of them is completely satisfactory. Often the healthy kidney compensates for the insufficiency of the diseased one to such a great extent that, if only the total function is measured, it is found to be normal and the state of the diseased kidney is masked in this way (163).

To study the functional capacity of the proximal tubules I used in the first place the renal extraction of PAH. Since the two kidneys are to be compared it is of minor importance whether or not the plasma concentration of PAH is kept on a low and constant level (4 mg/100 ml).

The function of the distal tubules and collecting tubules was studied by means of the renal concentration ability expressed as the highest osmolality of the urine determined as described in Chapter VII. However in view of the errors that are apt to arise in quantitative collection of urine during catheterization of a ureter I have not given

any quantitative data for the total excretion of individual electrolytes. Instead I have confined myself to giving the concentration in the specimens obtained from both ureters concurrently. The results of clearance tests are exceptions. The sources of error have in fact proved to be negligible in determinations of the osmolality of the urine by cryoscopy as well as in analyses of the urinary concentration of sodium and potassium by flame photometry (13, 117).

Obviously tubular function can be studied in other ways. On practical grounds I limited myself to the methods described except in a few cases in which determinations were made of the excretion of titratable acid and ammonia by each kidney separately. However since only a few cases were involved and the determinations were not made consistently no conclusions are permissible.

As already mentioned in Chapter III an additional reason for not including these determinations was that some patients have a tendency to metabolic acidosis which may easily develop from latent or manifest acidosis on loading with an acidifying salt. Urine heavily infected by urea splitting bacteria — e.g. some *Proteus* strains — is another complicating factor; ammonia is then released and gives false values for the concentration of both ammonia and titratable acid. When the pH of the urine was determined in connexion with passage of ureteric catheters it was impracticable for the test to be preceded by acidification. For this reason, reservations must be made for the

value of this determination. The pH values are nevertheless included in the tables and the results are compared with those of other selective renal function tests.

At our clinic, the *endogenous creatinine clearance* is used routinely for a study of the glomerular filtration rate. The creatinine is determined without any absorption procedure, *i.e.*, without taking into account the chromogens which, like creatinine, give a colour reaction with picric acid. It has been shown by several authors that the chromogens present in plasma result in the creatinine value being somewhat higher than that representing the true creatinine (118). It has also been demonstrated that when the creatinine concentration is high, some part is excreted through the tubules, and it seems probable that it can also be reabsorbed to some extent in the tubules (12). Consequently, the endogenous creatinine clearance, as determined in this investigation, is only an approximate measure of the glomerular filtration rate, and is associated with considerable sources of error. It appears likely that the discrepancies caused by these errors are symmetric in healthy kidneys, as shown *e.g.* by Haugen's results (94). Naturally, the error introduced by the chromogens in the plasma affects the clearance value for each kidney to the same extent.

This argument applies not only to determination of the endogenous creatinine clearance but also to analysis of the *creatinine concentration* in the urine from each kidney, which was also used to study the existence of any asymmetry of function.

In many of my cases the *inulin clearance* was also determined using current methods. This clearance value is in all probability, as close as we can come at present to the true glomerular filtration rate. Nevertheless it

is not absolutely unquestionable that the inulin clearance too does not differ, to some extent, from the true filtration value. As early as 1945, Lindahl & Josephson (150) demonstrated that certain kinds of inulin were not recovered quantitatively in the urine of man after iv injection. This might indicate that certain inulin preparations are metabolized to some extent in the organism. Balint (7) for example stated that he had found indications that inulin can be accumulated in the kidneys of dogs. Experiments now in progress at our hospital also suggest that inulin clearance is not always identical with the glomerular filtration rate.

In my series, the *renal plasma flow* was studied chiefly by means of PAH. Determination of the PAH clearance is obviously associated with the same sources of error as other determinations of the clearance by each kidney separately. The PAH clearance is known to be of no value for estimation of the renal plasma flow or renal blood flow in damaged kidneys, in which the renal extraction is reduced. In my cases however, the PAH concentration was always determined in blood from the renal vein as well which largely eliminated this source of error. Other sources of error nevertheless remain such as possible accumulation of the test substance in the renal parenchyma (7, 119, 122, 123) and passage of PAH into the erythrocytes (121). It can however be presumed that these sources of error are of little consequence.

It is obvious that *roentgenologic examination* of the kidneys — apart from being one of the foremost diagnostic aids — is also a handy, valuable method for studying the symmetry and asymmetry of renal function. In hardly any case in the present series

did there appear to be a discrepancy between the results of roentgenologic examination and the functional tests. Since the roentgenologic examinations served merely as a standard of reference for the symmetry of renal function their value will be only briefly mentioned here.

As a rule excretory pyelography gives a clear picture of the anatomy of the kidneys provided that renal function is such that a sufficient density of contrast medium can be obtained (cf Chap I). On the other hand excretory pyelograms of the kidneys are inferior to quantitative determinations of renal function for assessing the degree of functional impairment (52 66 67 274 cf Chap VI). This is particularly true of renal artery stenosis in which Morris & DeBailey (172) reported that 30 per cent of their patients presented normal pyelograms. The results in this category of patients in the present study largely agree with this observation.

The interval between pyelography and ureteric catheterization should be as short as possible one of the reasons being to establish whether there is any obstruction of the urinary tract.

Although determination of the *bacterial type and bacterial count* in the urine from each kidney is also of some diagnostic value these determinations have several technical limitations e.g. the interval before cultivation can begin (the number of bacterial colonies doubles in about 20 minutes) and the effect of anatomic obstructions on the urinary flow. A difference between the incidence of bacteria in the urine from the two kidneys cannot be said to have been established unless — in untreated cases — the bacterial count per unit volume of urine is at least 10 times greater on one side. The

borderline for significant bacteriuria has been drawn at 10 000 bacteria/ml urine. For bladder urine the corresponding value is 100 000 bacteria/ml (127 cf Chaps I and III). These limits serve as satisfactory screening tests but must be lowered if the patient is undergoing treatment.

Effect of Renal Vein Catheterization on the Determinations

When a comparison is to be made between the extraction by the two kidneys variations in the anatomic and topographic conditions may play a certain role.

If the catheter tip lies far out in the renal vein there is a risk that blood may be collected only from a limited part of the kidney — e.g. from a greatly damaged part of it from a relatively undamaged part or from a healthy part. If the renal damage is homogeneous this may not have any great effect on the symmetry of the values but if renal function is inhomogeneous the magnitude of this source of error cannot be estimated. In the present investigation I tried to avoid advancing the catheter tip so far that this source of error would come into effect.

On the other hand, Edvall (70) demonstrated that the catheter tip cannot lie just caudally or centrally in the renal vein without the risk of admixture of blood from the vena cava and the tributaries (testicular/ovarian and suprarenal veins) although the latter applies only to the left renal vein. Smith (232) stated in fact, that renal vein catheterization should not be performed on the left side in view of this likelihood of admixture. Edvall (70) showed that the catheter tip should be advanced at least 2 cm in the left renal vein to eliminate the

risk of mixing with blood from tributaries, as well as backflow from the vena cava. However, Johnstone (116), who made autopsy studies in 10 subjects with healthy kidneys, found that the distance between the opening of the suprarenal vein into the left renal vein and the vena cava was a mean 3.19 cm (range 2.0–4.5). In three cases the testicular/ovarian vein opened directly opposite the suprarenal vein and in four twice as far in. In the remaining three cases, the testicular/ovarian vein did not open into the renal vein. The mean distance from the site of opening to the vena cava was 5.09 cm (range 3–9).

The value for the PAH extraction by the left kidney without the contribution of blood from the tributaries can be calculated. For this calculation, the apparent extraction and the RPF in the relevant kidney must be known as well as the quantity of plasma from the tributaries. The blood flow from the left suprarenal vein was determined by Bucht (43) and found to be 2–5 ml/minute (mean in 5 healthy subjects 2.74 ml/minute). He estimated the blood flow from the ovarian/testicular vein to be approximately the same quantity.

The corrected extraction E_c , i.e., that without admixture of blood from the tributaries, can be calculated from the value for the PAH extraction (apparent extraction E_a) obtained in the usual way, according to the equation

$$E_c = E_a \frac{\text{RPF}}{\text{RPF} - b}$$

in which RPF is the renal plasma flow without correction for the tributaries and b is the quantity of plasma in the blood flowing in from the tributaries per minute. In view of the difficulty of estimating the latter quantity, I calculated for a possible admix-

ture of blood of both 5 and 10 ml/minute, i.e., 3 and 6 ml/minute of plasma, respectively (Table VIII).

The RPF could be measured in only 7 of the 35 cases of chronic and acute pyelonephritis in which the PAH extraction by each of the kidneys was determined. Consequently, the correction could not be made consistently in the comparison between right and left kidney. Table VIII shows the influence of the correction on the PAH extraction value of the left kidney in the 7 cases in which it could be calculated. The values were computed in relation to corresponding weighted clearance periods. They do not therefore, correspond exactly to the mean values for PAH extraction and clearance respectively by the left kidney listed in Table X, since the latter values were usually calculated as the mean of several periods. The only reason for presenting the calculations was, in fact, to show the extent to which any admixture of blood from the tributaries may affect the extraction values. It can be inferred from Table VIII that this correction changed the relevant values only inappreciably. Consequently the difference does not alter the conclusion that as a rule the PAH extraction by the left kidney is smaller than that by the right.

In my cases the catheter was advanced as far as possible in the left renal vein and then slowly withdrawn until a satisfactory flow was obtained on aspiration. The position of the tip was checked by fluoroscopy. The same procedure was used on the right side.

In the present investigation I catheterized the right and left renal vein at the same session in 11 healthy volunteers and determined the PAH extraction by each kidney. The results are listed in Table III from

Table VIII Corrected PAH extraction by left kidney (E_c , L) according to formula on p. 62 for hypothetical influence of blood from the tributaries on the extraction value

Age yr	Right kidney			Left kidney						
	C_{PAH}	E_a	RPF	C_{PAH}	E_a	RPF	E_c for		$E_c - E_a$ for	
	ml/min	/	ml/min	ml/min	%	ml/min	3 ml/min	6 ml/min	3 ml/min	6 ml/min
4a	73	46.9	156	70	37.4	187	38.15	38.52	0.75	1.12
8a	167	78.3	213	119	58.9	202	60.08	60.67	1.18	1.77
12	175	61.5	285	213	72.5	295	73.23	73.95	0.73	1.45
13a	132	80.6	164	110	82.5	133	84.15	86.63	1.65	4.13
19a	90	69.1	72	36	69.6	52	73.78	78.65	4.18	9.04
20a	92	85.3	108	55	55.0	100	56.65	58.30	1.65	3.30
22a	51	31.9	160	17	18.3	93	18.85	19.58	0.55	1.28

For comparison the PAH clearance by both kidneys is given, as well as the PAH extraction (E_a) and RPF calculated in the conventional way. The extraction is corrected for a plasma flow from the tributaries of both 3 and 6 ml/min.

The differences (E_c of renal extraction) between the hypothetical values and those calculated conventionally are listed in the last two columns.

which it can be inferred that the extraction was the same bilaterally. Since no significant difference was present between the right and left kidney in this respect it seems allowable to conclude that an admixture of blood from tributaries and from the caval vein does not play any essential role at any rate in healthy kidneys. It is true that the normal series in question consisted of young persons and that the mean age in the series of patients was much higher. It nevertheless seems unlikely that this age difference would have influenced the source of error which blood from the tributaries may imply.

Ofstad (181) also found that the sampling error due to intermixture of non renal blood is small at the maximum of the same order of magnitude as the sum of the chemical analysis errors in measurements of E_{PAH} and not different on the two sides.

Xylocaine[®] was used as a local anaesthetic when passing the catheter into the renal vein. Since this preparation is excited by non ionic diffusion it is highly improbable that it influenced the PAH extraction (76).

Despite all precautions I was not invariably able to avoid errors in determination of the renal extraction of PAH. This is evident from the fact that in a few cases in which more than two determinations were made from one side on the same occasion, one value differed considerably from the others. Obviously in such cases it cannot be ruled out that a transient change had occurred in the kidney's extraction ability. It nevertheless seems more probable that one or more of the aforementioned sources of error had taken effect. Which of them was responsible must remain an open question.

If an individual value showed an extremely large deviation (≥ 50 per cent of the mean value) it was however considered to be evident that the catheter tip had slipped so that the blood derived from the vena cava. The deviating value was then omitted from the calculation of the mean value. Such an omission was made in 5 cases (Table IX).

In case 128 with stenosis of the right main renal artery the mean value for PAH extraction by the left kidney was 5.8 per cent.

Table IX E_{PAH} values omitted
All the E_{PAH} values in the cases where the
bracketed values were omitted from Table X

Case no	Right	Left
2a	84.2 79.4 (23.1)	
14c	61.5 61.3 71.9 72.5 (6.7) 70.5 70.5 68.8	
84a		72.6 74.0 (7.4) 77.6 77.7
90a		12.1 11.7 (6.5) 9.3 35.3
117a	88.7 85.2 85.3 (12.3)	

This value was omitted from both the tables and the figures, since it was regarded as unquestionable that a technical error had been made. The extraction of PAH by the right kidney was 71.0 per cent, but the creatinine clearance by this kidney was lower than that by the left kidney. The osmolality of the urine and its sodium concentration were the same bilaterally.

In the tables, the cases in which one or several of the values for the renal extraction of PAH deviated from the mean by ≥ 25 per cent are marked with an asterisk beside the relevant value.

Effect of Ureteric Catheterization on the Determinations

As far as separate determinations of the clearances are concerned, I departed from the principle of using only methods which did not require complete collection of the urine. One of the reasons why this was regarded as permissible was that all clearances were determined during at least two consecutive periods. Nevertheless, it cannot be denied that a risk of erroneous results existed.

In the cases in the present series, it is evident that leakage of urine outside the ureteric catheter was an appreciable source of error in only a few cases. Thus when the urine from the bladder was to be collected separately through an indwelling catheter, none was as a rule obtained. However in three patients (cases 2, 5, 136) the leakage amounted to 30, 19 and 35 per cent, respectively, of the total diuresis. Their clearance values were therefore omitted from the tables and figures. In the other 34 examinations in which clearance determinations were made, the leakage — if any — was so slight that it could not be measured.

It has been suggested that the urine escaping into the bladder should be calculated in relation to the quantity of urine excreted per time unit by each kidney separately (51, 55, 145, 149, 187, 233). Such calculations are not however, to be recommended since it has been postulated that a difference is present between the urine which passes through the ureteric catheters and that which passes outside them with respect to composition and concentration (137). Thus no simple mathematical relation exists between the concentration of urine drained through the catheter and that escaping into the bladder. Hoeg (102) also criticized the aforementioned

methods for calculating the leakage which he estimated at 1.7 per cent. In addition, he pointed out that if the concentrations are identical such calculations cannot be made and if they differ slightly there is a wide range of uncertainty. Haugen *et al* (94) too emphasized the importance of leakage in bilateral clearance determinations by means of ureteric catheterization. Of 41 attempts at such determinations 16 were unsuccessful 6 of them on account of leakage.

For the reasons given in the foregoing I made no attempt to calculate how large a proportion of the urine collected in the bladder had leaked from the right and left ureteric catheter respectively.

With respect to unilateral *versus* bilateral catheterization Dustan *et al* (65) stated that there is no disadvantage in catheterizing only one ureter with an occluding catheter and collecting the urine from the other via the bladder. They found no support for the supposition that urine would leak undetected beside the ureteric catheter and mix with that from the other kidney collected in the bladder. Nor were they of the opinion that catheterization has any effect on renal function.

In my series unilateral ureteric catheterization was performed in only one patient (case 121) and the urine from the other side was collected from the bladder.

The results of Prat & Kocvara (196) were in opposition to the view of Dustan *et al* (65) i.e. that ureteric catheterization has little if any effect on renal function. The former authors stated that in most cases catheterization decreases the blood flow through the kidney and often causes a decrease in the glomerular filtration rate (GFR) as well. They ascribed the latter to

spasm of the vasa efferentia. Diuresis usually increases as a result of diminished reabsorption of water as also found by Schück & Hradec (223). In another publication Prat & Kocvara (197) reported that changes in renal function occurred in approximately the same extent irrespective of whether catheterization was uni- or bilateral. They stated that even introduction of a catheter into the bladder could increase diuresis although only inappreciably.

The experience of Larsen (145) and of Höeg (102) was in contrast to these observations. They found that the clearance values determined on bladder urine during the control period before passage of the catheters were in almost every case higher than the sum of the clearances in the urine drained through both ureteric catheters.

The effect of emotional factors on renal haemodynamics and function has also been studied by several authors e.g. by Brod & Sirota (32) who made experiments on dogs. Hinkle *et al* (100) found in man that stimuli in the form of strong intimidation increased diuresis by 200–300 per cent in hydropaenic patients with an associated decrease in specific gravity and chloride concentration in the urine. Miles & de Wardener (165) studied the emotional effect of catheterization in 10 normotensive and 10 hypertensive patients. Increased diuresis was recorded in all of the latter it was sometimes large and was caused mainly by a raised excretion of Na and chlorides. Nine of the 10 normotensive patients showed a slight increase in the urinary volume. The authors stated that the increased electrolyte output was probably to be ascribed to decreased tubular reabsorption rather than to an associated increase in GFR. They concluded that emotional factors may be res-

possible for an abnormally high excretion of electrolytes

Wolf (271) found that pain decreased the renal blood flow, and explained this observation on the basis of the view expressed by Chasis *et al* (50), *ie*, that the renal plasma flow is regulated by the efferent glomerular arterioles. This led Wolf to conclude that pain causes contraction of both the efferent and afferent arterioles with a resulting decrease in the flow of plasma through the kidney. Because of the increase in the filtration fraction, the efferent vessels must contract to a greater extent — in other words, the renal plasma flow decreases — whereas the glomerular filtration rate is maintained.

The possibility cannot be disregarded that a purely mechanical irritation may elicit the aforementioned vasospasm. Thus Hix (101) for example, stated that irritation of one ureter in the dog caused a decrease in renal plasma flow and glomerular filtration rate in this kidney. He found sensory nerves running from the ureter, which communicated reflexly with renal efferent constrictor fibres. As mentioned previously, Haugen *et al* (94) were unable in 16 of 41 patients, to perform bilateral clearance determinations by means of ureteric catheterization. In 5 of these cases, failure was due to the instrumentation causing such discomfort to the patient that the examination had to be discontinued.

Spasm is apt to occur around the catheter. Nevertheless, according to Kul (132), no reflexogenic disturbances occur even when there is negative pressure, despite adherence of the catheter tip to the ureteric mucosa.

An additional factor of consequence for the urinary volume is the effect of the anti diuretic hormone (ADH). Induction of

a ureteric catheter may — via the central nervous system — reduce the production of ADH, and thereby decrease the reabsorption of water (176). Verney (258) showed that water diuresis does not recommence immediately after the decrease in diuresis caused by ADH, but that 15–20 minutes elapse before the ADH circulating in the blood is eliminated. Consequently, the increased diuresis which I — like Prat & Kocvara (196) — sometimes observed during the first 5–15 minutes after passage of the catheter cannot be ascribed solely to suppression of ADH secretion. Therefore, when determining the GFR and osmolarity of catheter specimens, I always discarded this first portion of urine. De Wardener (263) described three types of emotional effect on diuresis. In the first type, diuresis is decreased due to an enhanced production of ADH as a result of pain, fear or similar reactions. The second type of reaction is osmotic diuresis. Finally, the third type is characterized by water diuresis caused by inhibition of ADH production.

It is thus impossible, in the individual case, to decide which of the aforementioned patterns — alone or in combination — plays the greatest role in altering the renal haemodynamics.

In order to evaluate the function of each kidney properly, it is important to have a basis of comparison in the form of a study of the combined activity of both kidneys made before the separate examination or in some cases after it. On technical grounds this requirement could not be fulfilled in every case in the present series.

In addition, I tried as far as possible to eliminate the disturbing factors that may be elicited by catheterization. One possibility

is to delay taking the specimens until 30—40 minutes after the catheters have been passed and the bladder emptied (196). This prolongation of the examination does however increase the risk of spasm around the catheters. For this reason, I generally confined myself to waiting until the urinary flow was relatively constant (usually 15—20 minutes). Every effort was made to prepare the patients psychologically and instrumentation was preceded by thorough local anaesthesia of the mucosa.

Figure 1 A and B illustrates that ureteric catheterization *per se* may constitute a source of error. It can be seen that, in some of my patients a higher value was obtained for the renal concentration ability when the test was made without catheterization than that obtained for the better kidney at catheterization. This implies that the concentration ability had decreased at any rate in the less involved kidney. It cannot be stated whether or not it was affected in the poorer kidney as well. This reduced renal concentration ability during ureteric catheterization is in agreement with the increased diuresis observed in this connexion by other authors cited in the foregoing.

A lack of agreement between the renal concentration ability during ureteric catheterization and that without instrumentation is not necessarily to be ascribed to a reflex mechanism to psychological factors or to pain caused by the instruments since the two examinations were made in an interval of 3—7 days. The state of the patient's kidneys may therefore have changed and the degree of dehydration may have differed. Figure 1 suggests that this source of error was of relatively little consequence at any rate in the cases of chronic and acute pyelonephritis.

It is a common experience in serial clearance determinations — or in other investigations in which quantitative collection of urine is an essential factor — that consecutive double determinations in the same individual may show poor agreement. In view of the sources of error associated with ureteric catheterization it is evident that such a lack of conformity between values from different periods may be still more frequent when dealing with the function of one kidney only. Thus did in fact apply to some patients in my series.

It would perhaps have been easier to draw conclusions from the results if those with poor agreement between the periods had been omitted. In most cases the discrepancy is probably to be ascribed to technical errors or accidents to the patient's subjective complaints or to other psychological factors. Nevertheless it cannot be ruled out that a spontaneous change had occurred in e.g. the glomerular filtration rate, diuresis and reabsorption of electrolytes during a short part of the investigation period. For this reason I did not omit any values. However since the mean values based on results with poor agreement between the periods are of little worth when conclusions are to be drawn, such deviations are noted in the tables. Thus mean values in which the result of one or more determinations differed by ≥ 50 per cent from the mean are marked with a circle (o). This limit was ≥ 25 per cent for EPAH and the relevant value is marked with an asterisk (cf. page 64). When making determinations on catheter specimens it does not seem permissible to omit a single value as unquestionably due to a technical error as was considered justified with respect to values for the renal extraction of PAH (Table IV).

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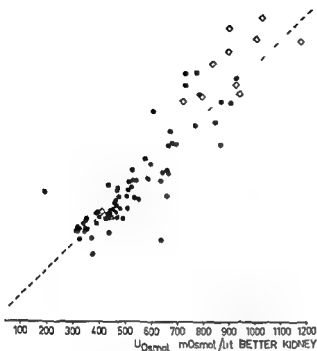
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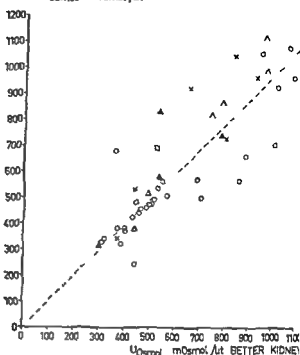
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U_{Osmol} mOsmol /lit



A Chronic and acute non obstructive pyelonephritis (groups A1 A2)

TOTAL U_{Osmol} mOsmol /lit



B Miscellaneous renal diseases (groups B1—B3)

Fig 1 Concentration ability (mOsmol/lit) of both kidneys (total) without catheterization (ordinate) and concentration ability of the better kidney (abscissa) — The broken line represents symmetric values For symbols see p 39

I did not investigate whether, in my cases, ureteric catheterization had influenced other renal functions than the concentration ability. Obviously, it cannot be excluded that the renal plasma flow, clearances PAH

extraction and glomerular filtration rate were also affected. Nor does my series allow any conclusions about whether or not the functional changes elicited by catheterization in several cases were symmetric.

CHAPTER IX

RESULTS

The results of the tests in which the function of each kidney was studied separately are given in Table X.

In most of the cases in groups A1 A2 B1 and B2 in which asymmetric renal function was observed all the parameters studied were asymmetric in the same direction *i.e.* one kidney functioned better than the other in every such respect This is demonstrated in Tables XI and XII

In the cases of stenosis of the main renal artery with arterial hypertension (group B3) the pattern differed from that in the aforementioned groups as can be inferred from Tables X XI and XII This pattern will be discussed in Chapter X (pp 114—116) As far as groups B4 and B5 are concerned it can be seen in Tables XI and XII that in the great majority of cases there was symmetry between the two kidneys as regards roentgenologic features bacteriologic culture of the urine tenderness to palpation over the loins and subjective complaints whereas more divergent results were noted in other tests

In the tables poorer function of the right kidney is indicated by an arrow pointing upwards (↑) and poorer function of the left kidney by an arrow pointing downwards (↓) The sign = denotes that the difference between the kidneys did not exceed the limits for symmetry of the various parameters given in Chapter I The sign [=] denotes that there was not only no difference between the relevant function of the two

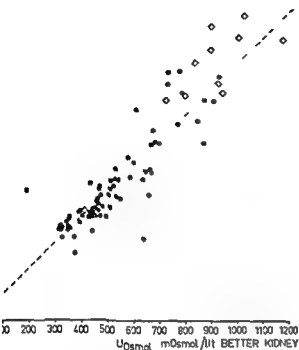
kidneys but also that both values lay within the normal range

An analysis of Table XI shows that in certain cases the asymmetry of one or several individual functions was in the opposite direction to that of the other functions Table XII therefore shows the number of investigations in which the parameters tested were symmetric those in which they were in agreement with the majority of the other bilateral investigations in being asymmetric and those in which the asymmetry was not consistent Finally it contains a group of cases denoted as indecisive since the comparative studies were either too few or gave too indecisive results to permit evaluation of which kidney had the better function

Since the PAH clearance is of little value in determinations in diseased kidneys these results have been omitted from Tables XI and XII The endogenous creatinine and inulin clearances are expressions of the same function they have therefore been counted as one test when both arrows pointed in the same direction In the few cases in which the arrows representing creatinine and inulin clearance pointed in the opposite direction that of the former has been taken as decisive since for practical reasons the creatinine clearance was determined in more cases than the inulin clearance

A few of the investigations in which the asymmetry of various parameters of renal function was inconsistent merit brief comment An example of a case in which the renal concentration ability differed from the other tests is the following

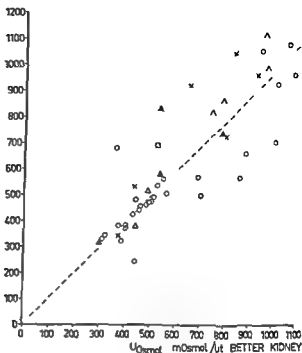
smol mOsmol /lit



A Chronic and acute non obstructive pyelonephritis (groups A1 A2)

Fig 1 Concentration ability (mOsmol/lit) of both kidneys (total) without catheterization (ordinate) and concentration ability of the better kidney (abscissa) — The broken line represents symmetric values For symbols see p 39

TOTAL UOsmol mOsmol /lit



B Miscellaneous renal diseases (groups B1--B5)

I did not investigate whether, in my cases, ureteric catheterization had influenced other renal functions than the concentration ability. Obviously, it cannot be excluded that the renal plasma flow, clearances, PAH

extraction and glomerular filtration rate were also affected. Nor does my series allow any conclusions about whether or not the functional changes elicited by catheterization in several cases were symmetric.

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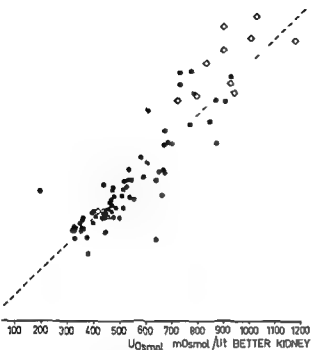
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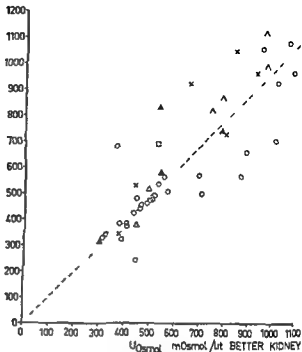
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U_{Osmol} mOsmol/lit



A Chronic and acute non obstructive pyelonephritis (groups A1 A2)

TOTAL U_{Osmol} mOsmol/lit



B Miscellaneous renal diseases (groups B1—B5)

Fig 1 Concentration ability (mOsmol/lit) of both kidneys (total) without catheterization (ordinate) and concentration ability of the better kidney (abscissa) — The broken line represents symmetric values For symbols see p 39

I did not investigate whether, in my cases, ureteric catheterization had influenced other renal functions than the concentration ability. Obviously, it cannot be excluded that the renal plasma flow clearances, PAH

extraction and glomerular filtration rate were also affected. Nor does my series allow any conclusions about whether or not the functional changes elicited by catheterization in several cases were symmetric.

Table 3a. Investigations of each kidney

Case no	5			6			7		
	U _{Cr} (mEq/lit)			U _{pH}			E _{PAH} ()		
	R	L	Diff	R	L	Diff	R	L	Diff
GROUP A1 Chronic non-obstructive pyelonephritis (≥ 3 cardinal criteria)									
1	—	—	—	—	—	—	8.2	74.4	-7.8
2a	—	—	—	—	—	—	81.8	64.1	-17.7
3	—	—	—	—	—	—	—	—	—
4a	—	—	-30	38	—	—	57.8	64.7	+6.4
4b	—	—	—	—	—	—	47.8	39.2	-8.6
5	—	—	—	—	—	—	74.7	73.6	-1.1
6b	—	—	—	—	—	—	—	—	—
6c	70	85	-15	31	—	—	75.6	60.1	-15.5
7a	—	—	—	—	—	—	89.4	71.0	-18.4
7b	—	—	—	—	—	—	—	—	—
8a	—	—	—	—	—	—	—	—	—
8b	—	—	—	—	—	—	79.0	79.7	-0.7
9a	—	—	—	—	—	—	63.0	54.6	-8.4
9b	65	83	-18	15	—	—	53.5	44.8	-8.7
10	—	—	—	—	—	—	8.8	74.0	-65.2
11	—	—	—	—	—	—	86.6	82.6	-4.0
12	—	—	—	—	—	—	—	—	—
13a	—	—	—	—	—	—	17.5	14.7	-2.8
13b	—	—	—	—	—	—	60.7	72.5	-11.8
14a	—	—	—	—	—	—	81.6	8.3	+73.3
14b	—	—	—	—	—	—	90.4	88.6	-1.8
14c	—	—	—	—	—	—	49.0	38.2	-10.8
15	—	—	—	—	—	—	—	—	—
16	50	80	+30	38	—	—	68.1	63.2	-4.9
17	—	—	—	—	—	—	75.5	64.3	-11.2
18a	—	—	—	—	—	—	—	—	—
18b	—	—	—	—	—	—	53.6	87.7	-34.1
19a	—	—	—	—	—	—	76.4	37.7	+38.7
19b	—	—	—	—	—	—	34.7	19.6	+15.1
20a	—	—	—	—	—	—	69.4	70.0	-0.6
20b	—	—	—	—	—	—	88.4	69.2	-19.2
21	—	—	—	—	—	—	86.2	39.7	+46.5
22a	—	—	—	—	—	—	—	—	—
22b	—	—	—	—	—	—	—	—	—
23a	—	—	—	—	—	—	—	—	—
23b	—	—	—	—	—	—	16.0	21.9	-5.9
24	—	—	—	—	—	—	65.3	58.9	-6.4
25a	—	—	—	—	—	—	—	—	—
25b	—	—	—	—	—	—	—	—	—
26	—	—	—	—	—	—	—	—	—
27	—	—	—	—	—	—	—	—	—
28a	—	—	—	—	—	—	92.4	87.2	-5.2
28b	—	—	—	—	—	—	70.4	44.5	+25.9
29a	—	—	—	—	—	—	79.8	71.8	-8.0
29b	—	—	—	—	—	—	83.7	79.8	-3.9
30	—	—	—	—	—	—	95.9	96.0	-0.1
31	—	—	—	—	—	—	—	—	—
GROUP A2 Acute and suspected chronic non-obstructive pyelonephritis (< 3 cardinal criteria)									
70	—	—	—	—	—	—	83.8	89.5	-5.7
71	—	—	—	—	—	—	86.1	83.3	+2.8
72	—	—	—	—	—	—	87.3	88.2	-0.9

Col. nos 1

a, b, c and d denote examinations on different occasions in the same patient.

Foot-note 1

See column 16

Col. nos 2

The test was made without dehydration or administration of Piresin® when the relevant value is marked by

Column 7

When one or more of the E_{PAH} values deviated from the mean by ≥ 25%, the relevant value is marked with an asterisk. The cases in which the values were omitted are listed in Table IX.

Table Xa Investigations of each kidney

Case no	2				3				4		
	Max conc ability (mOsmol/lit)				UNa (mEq/lit)				U _K (mEq/lit)		
	R	L	Dif	/	R	L	Dif	/	R	L	Dif
GROUP A1 Chronic non obstructive pyelonephritis (≥ 3 cardinal criteria)											
1	535	505	- 30	6	80	58	-22	28	-	-	-
2a	409	426	+ 17	4	-	-	-	-	-	-	-
2b	471	500	+ 29	6	-	-	-	-	-	-	-
3	545	401	-140	26	146	130	-16	11	-	-	-
4a	326	230	- 96	29	-	-	-	-	-	-	-
4b	430	410	- 20	5	128	121	- 7	5	-	-	-
5	339	271	- 68	20	-	-	-	-	-	-	-
6b	460	405	- 55	12	-	-	-	-	-	-	-
6c	465	440	- 25	5	78	92	+14	15	-	-	-
7a	425	488	+ 63	13	-	-	-	-	-	-	-
7b	512	523	+ 11	2	138	148	+10	7	-	-	-
8a	430	414	- 16	4	-	-	-	-	-	-	-
8b	-	-	-	-	-	-	-	-	-	-	-
9a	313	300	- 13	4	-	-	-	-	-	-	-
9b	507	491	- 16	3	-	-	-	-	-	-	-
10	617	763	+136	III	-	-	-	-	-	-	-
11	370	343	- 27	7	-	-	-	-	-	-	-
12	265	376	+111	30	-	-	-	-	-	-	-
13a	500	437	- 63	13	-	-	-	-	-	-	-
13b	572	577	+ 5	1	360	348	-12	3	-	-	-
14a	505	495	- 10	2	-	-	-	-	-	-	-
14b	465	460	- 5	1	-	-	-	-	-	-	-
14c	560	655	+ 95	15	81	50	-11	11	-	-	-
15	441	379	- 62	14	-	-	-	-	-	-	-
16	377	477	+100	21	106	110	+ 4	4	-	-	-
17	355	395	+ 40	10	-	-	-	-	-	-	-
18	398	368	- 30	8	-	-	-	-	-	-	-
19a	306	390	+ 84	22	-	-	-	-	-	-	-
19b	360	465	+105	23	18	43	+25	58	-	-	-
20a	438	286	-152	35	-	-	-	-	-	-	-
20b	655	330	-325	50	-	-	-	-	-	-	-
21	287	218	- 69	24	75	72	- 3	4	22.6	14.2	8.4
22a	318	229	- 89	28	-	-	-	-	-	-	-
22b	-	258	-	-	25	33	+ 8	24	-	-	-
23	670	915	+245	27	198	228	+30	13	-	-	-
24	318	355	+ 37	10	-	-	-	-	-	-	-
25a	265	440	+175	40	-	-	-	-	-	-	-
25b	393	455	+ 62	14	118	120	+ 2	2	-	-	-
26b	333	433	+100	23	-	-	-	-	-	-	-
27	777	717	- 60	8	-	-	-	-	-	-	-
28a	-	-	-	-	-	-	-	-	-	-	-
29c	-	-	-	-	-	-	-	-	-	-	-
30b	-	-	-	-	-	-	-	-	-	-	-
31	-	-	-	-	-	-	-	-	-	-	-
GROUP A2 Acute and suspected chronic non obstructive pyelonephritis (< 3 cardinal criteria)											
70	680	893	+203	23	-	-	-	-	-	-	-
71	671	714	+ 43	6	-	-	-	-	-	-	-
72	965	1013	+ 48	5	220	240	+ 20	8	-	-	-

The differences between the right and left kidney are given both in absolute figures (where + denotes that the right kidney has the higher value) and in % of the value for the better kidney (The differences in C_{PASH} have been omitted)

When one or more of the values obtained at ureter catheterization deviated from the mean by $\geq 50\%$ the relevant value is marked by

Symbols

- 0 Nothing pathologic observed or answer to question negative
- + Pathologic finding or answer to question positive
- (+) Doubtful positive finding or doubtful positive answer
- Not investigated or questioned

Table 2a (continued)

11				1		11		14		15		16	
Case no	C ₁₀ (ml/min)			Urine culture (bact/ml)		Roentgenol exam		Loin tenderness to palp		History of loin pain			
	R	L	Diff	R	L	R	L	R	L	R	L		
GROUP A1 Chronic non-obstructive pyelonephritis (≥ 3 cardinal criteria)													
1	-	-	-	-	2 ml.	0	(+)	+	0	0	(+)	0	
2	-	-	-	-	-	0	+	(+)	0	0	0	0	
3	-	-	-	-	0	80 000	0	+	0	0	0	0	
4a	18	14	-4	22	0	300 000	+	+	0	0	+	+	
4b	-	-	-	-	1 ml.	300 000	(+)	+	0	0	0	0	
5	-	-	-	-	-	-	-	-	0	0	0	0	
6a	-	-	-	-	3 000	230 000	(+)	+	+	0	0	0	
6b	-	-	-	-	0	0	(+)	+	+	0	0	0	
7a	-	-	-	-	0	0	0	+	0	0	0	0	
7b	-	-	-	-	5 000	5 000	0	0	0	0	0	0	
8	36	31	-5	14	-	-	0	0	0	0	0	0	
8b	-	-	-	-	-	-	0	+	0	0	0	0	
9a	13	8	5	38	50 000	50 000	0	+	0	0	0	0	
9b	-	-	-	-	0	0	(+)	+	0	0	0	0	
10	-	-	-	-	-	-	0	0	0	0	0	0	
11	5	7	+2	29	-	0	0	+	+	+	+	+	
12	41	32	+9	3	5 000	5 000	(+)	+	0	0	0	0	
13a	38	34	4	10	-	-	(+)	+	0	0	0	0	
13b	-	-	-	-	0	0	(+)	+	0	0	0	0	
14a	-	-	-	-	50 000	400 000	(+)	+	0	0	-	-	
14b	-	-	-	-	-	-	(+)	+	0	0	+	+	
14c	-	-	-	-	0	0	+	+	0	0	+	+	
15	-	-	-	-	-	-	0	+	0	+	+	+	
16	-	-	-	-	>100 000	0	+	(+)	0	0	-	-	
17	-	-	-	-	1 500	80 000	(+)	+	0	0	+	(+)	
18	-	-	-	-	1 100	0	+	+	+	0	0	0	
19a	15	11	4	27	-	-	+	(+)	0	0	0	0	
19b	-	-	-	-	100	100	(+)	+	0	0	0	0	
20a	29	19	10	34	50 000	50 000	(+)	+	0	0	0	0	
20b	-	-	-	-	400 000	140 000	(+)	+	0	0	0	0	
21	-	-	-	-	0	5 000	(+)	+	0	0	+	+	
22a	1	5	-4	38	-	-	+	+	+	0	0	0	
22b	-	-	-	-	50	0	(+)	+	0	0	0	0	
23	-	-	-	-	50 000	800 000	(+)	+	0	0	0	0	
24	-	-	-	-	>100 000	0	+	+	0	0	0	0	
25a	-	-	-	-	15 000	6 000	+	(+)	0	0	+	+	
25b	-	-	-	-	-	-	+	+	0	0	+	+	
26	-	-	-	-	-	-	(+)	0	0	(+)	0	0	
27	-	-	-	-	0	0	+	+	0	0	+	+	
28a	-	-	-	-	0	0	+	+	0	0	+	+	
28b	-	-	-	-	50 000	>100 000	+	(+)	+	0	+	+	
29	-	-	-	-	-	-	+	+	0	0	0	0	
30	-	-	-	-	-	-	0	0	+	0	0	0	
31	-	-	-	-	-	-	+	0	+	0	0	0	
GROUP A2 Acute and suspected chronic non-obstructive pyelonephritis (< 3 cardinal criteria)													
70	-	-	-	-	0	0	0	0	0	0	+	0	
71	-	-	-	-	0	0	0	0	0	0	+	0	
72	-	-	-	-	135	0	0	0	0	0	+	0	

E_D (%)

R	L	Diff
43.4	44.1	-1.1

* Am chlor load

U_{Cr} (mEq/lit)

R	L	Diff
78	71	-7

E_D (%)

R	L	Diff
26.1	16.5	-10.0
37.8	31.1	-6.7

E_D (%)

R	L	Diff
43.6	24.8	25.8

Table Xa (continued)

[illegible]

Table Xa (continued)

Case no	5			6			7		8		9		10	
	UCr (mEq/lit)			U _{PH}			Urine culture (bact/ml)		Roentgenol exam		Loin tenderness (to palp)		History of loin pain	
	R	L	D#	R	L	D#	R	L	R	L	R	L	R	L
GROUP A1 Chronic non-obstructive pyelonephritis (≥ 3 cardinal criteria)														
3	550	450	+10	15	—	—	1.2 mill.	600 000	+	+	0	+	—	+
33	—	—	—	—	—	—	0	0	+	+	0	+	—	—
34	—	—	—	—	—	—	70	300 000	(+)	—	+	+	—	—
35	60	70	+10	14	—	—	0	0	—	—	+	+	—	—
36	—	—	—	—	—	—	—	—	0	0	0	0	—	—
37	110	10	+10	8	—	—	0	0	0	0	0	+	+	(+)
38	75	40	-23	33	—	—	0	200 000	(+)	+	0	+	—	—
39	—	—	—	—	—	—	0	0	(+)	+	0	+	—	—
40	—	—	—	—	—	—	>100 000	0	+	+	+	+	—	—
41	—	—	—	—	—	—	—	—	+	+	0	0	—	—
43	—	90	3	3	776	749	60 000	—	+	+	0	0	—	—
44	80	90	+10	11	595	568	70	0	0	+	+	+	—	—
45	—	—	—	—	—	—	—	0	+	(+)	0	0	—	—
46	—	—	—	—	—	—	—	—	+	0	0	0	—	—
47	—	—	—	—	—	—	—	—	—	—	—	—	—	—
48	100	15	+3	2	609	605	1 mill.	3 mill.	(+)	(+)	0	+	+	+
49	—	—	—	—	667	617	0	0	0	0	0	0	—	—
50	90	40	-40	44	—	—	0	0	0	0	+	+	+	+
51	104	44	31	49	585	694	—	—	(+)	(+)	0	0	—	—
52	130	110	-0	15	51	61	0	25 000	(+)	+	0	0	—	—
53	140	130	-10	7	779	769	0	0	(+)	+	0	0	—	—
54	—	—	—	—	—	—	—	—	0	0	+	+	—	—
55	100	140	+40	79	—	—	50 000	30 000	(+)	+	+	+	—	—
56	88	78	10	11	575	575	0	0	0	+	+	+	—	—
57	100	100	0	0	—	—	4 mill.	4 mill.	(+)	(+)	+	+	—	—
58	0.7	0.8	+0.1	13	726	787	40 mill.	900	0	(+)	0	0	—	—
59	—	—	—	—	—	—	5 000	0	+	+	+	+	—	—
60	—	—	—	—	—	—	0	0	(+)	+	+	+	—	—
61	—	—	—	—	—	—	—	—	—	—	—	—	—	—
62	—	—	—	—	—	—	5 000	50 000	+	(+)	(+)	0	+	+
63	—	—	—	—	—	—	0	60 000	0	0	0	+	—	—
64	—	—	—	—	—	—	250 000	300 000	+	+	0	0	—	—
65	—	—	—	—	—	—	0	0	+	+	0	0	—	—
66	—	—	—	—	—	—	0	4 000	+	+	+	+	—	—
67	150	160	10	6	—	—	600 000	1 mill.	0	+	0	0	—	—
68	13	137	1	9	—	—	0	0	0	0	0	+	—	—
69	—	—	—	—	—	—	0	0	0	0	0	0	—	—
GROUP A2 Acute and suspected chronic non-obstructive pyelonephritis (< 3 cardinal criteria)														
73	—	—	—	—	—	—	—	—	—	—	—	—	—	—
74	—	—	—	—	—	—	—	—	—	—	—	—	—	—
75	90	—	30	33	—	—	1 000	400 000	0	0	0	0	0	0
76	—	—	—	—	—	—	0	0	0	0	0	0	—	—
77	—	—	—	—	—	—	0	0	0	0	0	0	—	—
78	80	60	70	25	—	—	0	0	0	+	0	0	—	—
79	135	150	+15	10	—	—	0	0	0	+	0	0	—	—
80	110	90	0	88	—	—	0	0	+	0	0	0	—	—

Table Xa (continued)

Case no	2				3				4		
	Max conc ability (mOsmol/lit)				UNa (mEq/lit)				UK (mEq/lit)		
	R	L	Diff	/	R	L	Diff		R	L	Diff
GROUP A1 Chronic non obstructive pyelonephritis (≥ 3 cardinal criteria)											
32	438	450	+ 12	3	128	123	- 5	4	-	-	-
33	702	834	+131	16	221	246	+25	10	-	-	-
34	663	603	- 60	9	107	104	- 3	3	-	-	-
35	340	351	+ 11	3	88	81	- 7	5	-	-	-
36	396	399	+ 3	1	-	-	-	-	-	-	-
37	663	673	+ 10	1	189	200	+11	6	-	-	-
38	517	410	-107	21	100	63	-37	37	-	-	-
39	350	300	- 50	14	13	13	0	0	-	-	-
40	305	250	- 55	18	-	-	-	-	-	-	-
41	560	643	+ 83	13	-	-	-	-	-	-	-
42	575	690	+115	17	130	132	+ 2	2	-	-	-
43	352	463	+111	24	99	144	+45	31	-	-	-
44	233	318	+ 85	27	86	104	+18	17	-	-	-
45	605	722	+117	16	-	-	-	-	-	-	-
46	323	250	- 73	23	-	-	-	-	-	-	-
47	463	470	+ 7	1	-	-	-	-	-	-	-
48	740	670	- 70	7	177	183	+ 6	3	-	-	-
49	735	765	+ 30	4	174	192	+18	9	-	-	-
50	635	322	-313	49	111	71	-40	36	-	-	-
51	505	348	-157	31	-	-	-	-	-	-	-
52	395	440	+45	26	140	142	+ 2	2	-	-	-
53	705	858	+153	18	152	168	+16	10	-	-	-
54	380	440	+ 60	14	80	80	0	0	-	-	-
55	690	740	+ 50	4	166	207	+41	20	-	-	-
56	452	405	- 47	10	88	84	- 4	3	-	-	-
57	663	653	- 10	2	168	168	0	0	-	-	-
58	555	858	+303	35	195	240	+45	19	-	-	-
59	345	352	+ 7	2	-	-	-	-	-	-	-
60	460	394	- 66	14	99	98	- 1	1	27	23	-4
61	393	364	- 29	7	-	-	-	-	-	-	-
62	630	395	-235	37	-	-	-	-	-	-	-
63	460	578	+118	20	-	-	-	-	-	-	-
64	525	240	-285	54	-	-	-	-	-	-	-
65	188	163	- 25	13	-	-	-	-	-	-	-
66	530	485	- 45	8	-	-	-	-	-	-	-
67	660	622	- 38	6	100	88	-12	12	-	-	-
68	893	863	- 30	3	-	-	-	-	-	-	-
69	340	349	+ 9	3	42	47	+ 5	11	-	-	-
GROUP A2 Acute and suspected chronic non-obstructive pyelonephritis (< 3 cardinal criteria)											
73	855	915	+ 60	7	201	204	+ 3	1	-	-	-
74	840	820	- 20	0	-	-	-	-	-	-	-
75	785	700	- 85	11	231	198	- 33	14	-	-	-
76	765	930	+165	18	137	103	- 34	25	-	-	-
77	882	793	- 89	10	175	173	- 2	0	20	27	-7
78	404	394	- 10	2	76	90	+ 14	16	-	-	-
79	775	990	+215	22	258	282	+ 24	9	-	-	-
80	1161	1153	- 8	1	285	270	- 15	5	-	-	-

Table Xb Investigations of each kidney
Symbols as in Table Ya

Symptoms as in Table 1										
1	5			6			7			
Case no	UCr (mEq/h)			UPH			EPH (Δ)			
	R	L	Diff	R	L	Diff	R	L	Diff	
GROUP B1 Chronic pyelonephritis with signs of urinary tract obstruction (≥ 3 cardinal criteria)										
81	-	-	-	-	-	-	730	693	-57	8
84	-	-	-	-	-	-	-	-	-	-
85	-	-	-	-	-	-	-	-	-	-
86	40	45	5	10	74	77	+0.3	-	-	-
87	8	30	+10	33	6.2	631	+0.09	97	368	+71
88	-	-	-	-	-	-	-	619	753	+56
89	-	-	-	-	-	-	-	160	55	-08
90	-	-	-	-	-	-	-	644	714	+70
93	-	-	-	-	-	-	-	-	-	10
96b	-	-	-	-	-	-	-	729	703	-26
96c	-	-	-	-	-	-	-	269	277	+08
97	-	-	-	-	-	-	-	940	961	+21
98	-	-	-	-	-	-	-	-	-	2
99	-	-	-	-	-	-	-	-	-	-
99c	90	85	-5	6	-	-	-	370	397	-473
99d	-	-	-	-	-	-	-	-	-	EM
99e	-	-	-	-	-	-	-	348	171	-177
99f	-	-	-	-	-	-	-	498	4.8	-70
99g	-	-	-	-	-	-	-	881	9	-789
99h	135	65	70	52	667	78	+0.61	-	-	51
99i	-	-	-	-	-	-	-	-	-	34
99j	-	-	-	-	-	-	-	-	-	90
99k	-	-	-	-	-	-	-	-	-	-
GROUP B2 Renal disease (except pyelonephritis) with unilateral or mainly asymmetric involvement										
106	-	-	-	-	475	515	+0.40	6.9	579	-50
107	-	-	-	-	-	-	-	860	1.0	740
108	-	-	-	-	-	-	-	654	737	+78
109	-	-	-	-	-	-	-	876	831	-45
110	-	-	-	-	-	-	-	397	681	+284
111	-	-	-	-	-	-	-	897	863	-34
112	31	39	+8	21	-	-	-	567	315	-252
113	-	-	-	-	-	-	-	-	-	67
GROUP B3 Arterial hypertension with stenosis or aneurysm of the main renal artery										
117a	8	10	+8	40	-	-	-	864	812	-52
117b	-	-	-	-	-	-	-	714	749	+35
118	-	-	-	-	-	-	-	765	8.4	+61
119	-	-	-	-	-	-	-	991	941	-50
120	-	-	-	-	-	-	-	8.9	871	08
121	-	-	-	-	-	-	-	890	840	-50
122	40	70	70	40	-	-	-	910	909	-01
123	-	-	-	-	-	-	-	881	834	-47
124	-	-	-	-	-	-	-	347	958	+611
125	-	-	-	-	-	-	-	907	880	-27
126	-	-	-	-	-	-	-	-	-	3
127	-	-	-	-	-	-	-	90	874	-28
128	-	-	-	-	-	-	-	889	848	+39
129	-	-	-	-	-	-	-	881	857	-24
130	-	-	-	-	-	-	-	-	-	-
GROUP B4 Renal disease with expected homogeneous involvement of both kidneys										
131	-	-	-	-	-	-	-	917	94	-35
132	-	-	-	-	-	-	-	877	846	-31
133	-	-	-	-	-	-	-	756	661	-95
134	-	-	-	-	57	57	8	746	7.9	-17
135	-	-	-	-	-	-	-	8.9	869	+40
136	-	-	-	-	455	490	+0.05	188	773	+585
137	-	-	-	-	-	-	-	881	893	+12
138	-	-	-	-	-	-	-	90	900	0
139	-	-	-	-	-	-	-	-	-	-
140	-	-	-	-	-	-	-	-	-	-
141	-	-	-	-	-	-	-	837	8.1	-16
142	-	-	-	-	-	-	-	937	7	-93
143	-	-	-	-	-	-	-	859	766	-93
GROUP B5 Patients with symptoms from the lower urinary tract in whom involvement of the kidneys could not be ruled out										
144	-	-	-	-	535	4.0	-0.05	87	908	+36
145	-	-	-	-	-	-	-	817	883	+66
146	-	-	-	-	-	-	-	875	885	+10

Table Xb Investigations of each kidney
Symbols as in Table Xa

Case no	2				3				4			
	Max conc ability (mOsmol/lit)				UNa (mEq/lit)				U _h (mEq/lit)			
	R	L	Diff	/	R	L	Diff		R	L	Diff	
GROUP B1 Chronic pyelonephritis with signs of urinary tract obstruction (≥ 3 cardinal criteria)												
81	452	434	- 18	4	-	-	-	-	-	-	-	-
82a	302	307	+ 5	2	-	-	-	-	-	-	-	-
82b	397	390	- 7	2	-	-	-	-	-	-	-	-
82c	480	423	- 57	12	142	120	-22	15	-	-	-	-
82d	540	335	-205	38	53	54	+ 1	2	-	-	-	-
83b	319	321	+ 2	1	-	-	-	-	-	-	-	-
84a	300	400	+100	25	-	-	-	-	-	-	-	-
84b	-	-	-	-	-	-	-	-	-	-	-	-
85	515	880	+365	41	-	-	-	-	-	-	-	-
86b	427	383	- 44	10	-	-	-	-	-	-	-	-
86c	510	437	- 73	14	-	-	-	-	-	-	-	-
87	215	441	+206	47	-	-	-	-	-	-	-	-
88	1003	668	-335	33	-	-	-	-	-	-	-	-
89	400	698	+298	43	-	-	-	-	-	-	-	-
4c	561	465	- 96	17	130	97	-33	25	-	-	-	-
90a	302	214	- 88	39	-	-	-	-	-	-	-	-
90b	-	-	-	-	-	-	-	-	-	-	-	-
91	-	-	-	-	-	-	-	-	-	-	-	-
92	883	375	-508	45	198	198	0	0	-	-	-	-
93	360	310	- 50	14	-	-	-	-	-	-	-	-
GROUP B2 Renal disease (except pyelonephritis) with unilateral or mainly asymmetric involvement												
106	547	915	+368	40	-	-	-	-	-	-	-	-
107	534	457	- 77	14	-	-	-	-	-	-	-	-
108	433	408	- 25	1	-	-	-	-	-	-	-	-
109	-	-	-	-	-	-	-	-	-	-	-	-
110	-	-	-	-	-	-	-	-	-	-	-	-
111	-	-	-	-	-	-	-	-	-	-	-	-
112	-	-	-	-	-	-	-	-	-	-	-	-
GROUP B3 Arterial hypertension with stenosis or aneurysm of the main renal artery												
117a	478	404	- 74	15 ¹	130	140	+10	7	63	41	-22	35
117b	357	365	+ 8	2 ¹	46	61	+ 5	8	86	82	- 4	5
118	622	457	-165	27	-	-	-	-	-	-	-	-
119	215	235	+ 20	9 ¹	53	48	- 5	47	16	16	- 10	38
120	614	564	- 50	8 ¹	130	159	+29	18	100	111	+ 9	39
121	262	234	- 28	11	93	116	+23	10	-	-	-	-
122	250	290	+ 40	14 ¹	91	82	- 9	39	39	32	- 7	15
123	438	444	+ 6	1	68	71	+ 3	4	5	5	-	11
124	375	188	-187	50 ¹	36	63	+27	43	34	8	- 25	74
125	287	261	- 26	9	80	111	+ 2	2	19	15	- 4	21
126	278	349	+ 71	20	88	86	- 2	2	23	11	- 12	0
127	556	536	- 20	4	176	160	+16	21	68	47	- 21	11
128	191	186	- 5	3	69	71	+ 2	3	10	9	- 1	10
129	-	-	-	-	-	-	-	-	-	-	-	-
GROUP B4 Renal disease with expected homogeneous involvement of both kidneys												
132	379	341	- 38	10	124	112	-12	10	27	25	- 2	7
133	190	289	+ 99	34	34	40	+ 6	15	33	25	+ 12	48
134	-	-	-	-	-	-	-	-	-	-	-	-
136	504	534	+ 30	6	-	-	-	-	-	-	-	-
137	744	780	+ 36	5	-	-	-	-	-	-	-	-
138a	475	460	- 15	3	-	-	-	-	-	-	-	-
139	1030	1125	+ 95	8	-	-	-	-	-	-	-	-
140	-	-	-	-	-	-	-	-	-	-	-	-
141	-	-	-	-	-	-	-	-	-	-	-	-
142	-	-	-	-	-	-	-	-	-	-	-	-
143	-	-	-	-	-	-	-	-	-	-	-	-
GROUP B5 Patients with symptoms from the lower urinary tract in whom involvement of the kidneys could not be ruled out												
148	570	522	- 48	0	-	-	-	-	-	-	-	-
149	779	759	- 20	3	166	168	+ 2	-	-	-	-	-
150	727	735	+ 8	1	-	-	-	-	-	-	-	-

Table Xb (continued)

		11			12		13		14		15		16	
Case no	Cln (ml min)			Urine culture (bact ml)		Roentge nol exam		Loin tender ness to palp		History of loin pain				
	R	L	Dff	R	L	R	L	R	L	R	L			
GROUP B1 Chronic pyelonephritis with signs of urinary tract obstruction (≥ 3 cardinal criteria)														
47	27	16	11	41	—	(+)	(+)	0	0	—	—			
48a	—	1	—	20	500 000	(+)	(+)	0	0	—	—			
48b	—	—	—	—	0	(+)	(+)	0	0	0	0			
48c	—	—	—	—	15 000	(+)	(+)	0	0	0	0			
48d	—	—	—	—	—	(+)	(+)	0	0	0	0			
48b	9	15	+ 6	40	50 000	(+)	(+)	0	0	0	0			
48a	7	15	+ 3	50	0	(+)	(+)	0	0	0	0			
48b	—	—	—	—	0	(+)	(+)	0	0	0	0			
48c	—	—	—	—	0	(+)	(+)	0	0	0	0			
48d	—	—	—	—	0	(+)	(+)	0	0	0	0			
48e	—	—	—	—	0	(+)	(+)	0	0	0	0			
48f	—	—	—	—	0	(+)	(+)	0	0	0	0			
48g	—	—	—	—	0	(+)	(+)	0	0	0	0			
48h	—	—	—	—	0	(+)	(+)	0	0	0	0			
48i	—	—	—	—	0	(+)	(+)	0	0	0	0			
48j	—	—	—	—	0	(+)	(+)	0	0	0	0			
48k	—	—	—	—	0	(+)	(+)	0	0	0	0			
48l	—	—	—	—	0	(+)	(+)	0	0	0	0			
48m	—	—	—	—	0	(+)	(+)	0	0	0	0			
48n	—	—	—	—	0	(+)	(+)	0	0	0	0			
48o	—	—	—	—	0	(+)	(+)	0	0	0	0			
48p	—	—	—	—	0	(+)	(+)	0	0	0	0			
48q	—	—	—	—	0	(+)	(+)	0	0	0	0			
48r	—	—	—	—	0	(+)	(+)	0	0	0	0			
48s	—	—	—	—	0	(+)	(+)	0	0	0	0			
48t	—	—	—	—	0	(+)	(+)	0	0	0	0			
48u	—	—	—	—	0	(+)	(+)	0	0	0	0			
48v	—	—	—	—	0	(+)	(+)	0	0	0	0			
48w	—	—	—	—	0	(+)	(+)	0	0	0	0			
48x	—	—	—	—	0	(+)	(+)	0	0	0	0			
48y	—	—	—	—	0	(+)	(+)	0	0	0	0			
48z	—	—	—	—	0	(+)	(+)	0	0	0	0			
49a	15	3	12	80	5 000	(+)	(+)	0	0	+	+			
49b	—	—	—	—	>100 000	(+)	(+)	0	0	+	+			
49c	—	—	—	—	>100 000	(+)	(+)	0	0	+	+			
49d	—	—	—	—	0	(+)	(+)	0	0	+	+			
49e	—	—	—	—	0	(+)	(+)	0	0	+	+			
49f	—	—	—	—	0	(+)	(+)	0	0	+	+			
49g	—	—	—	—	0	(+)	(+)	0	0	+	+			
49h	—	—	—	—	0	(+)	(+)	0	0	+	+			
49i	—	—	—	—	0	(+)	(+)	0	0	+	+			
49j	—	—	—	—	0	(+)	(+)	0	0	+	+			
49k	—	—	—	—	0	(+)	(+)	0	0	+	+			
49l	—	—	—	—	0	(+)	(+)	0	0	+	+			
49m	—	—	—	—	0	(+)	(+)	0	0	+	+			
49n	—	—	—	—	0	(+)	(+)	0	0	+	+			
49o	—	—	—	—	0	(+)	(+)	0	0	+	+			
49p	—	—	—	—	0	(+)	(+)	0	0	+	+			
49q	—	—	—	—	0	(+)	(+)	0	0	+	+			
49r	—	—	—	—	0	(+)	(+)	0	0	+	+			
49s	—	—	—	—	0	(+)	(+)	0	0	+	+			
49t	—	—	—	—	0	(+)	(+)	0	0	+	+			
49u	—	—	—	—	0	(+)	(+)	0	0	+	+			
49v	—	—	—	—	0	(+)	(+)	0	0	+	+			
49w	—	—	—	—	0	(+)	(+)	0	0	+	+			
49x	—	—	—	—	0	(+)	(+)	0	0	+	+			
49y	—	—	—	—	0	(+)	(+)	0	0	+	+			
49z	—	—	—	—	0	(+)	(+)	0	0	+	+			
50a	—	—	—	—	0	(+)	(+)	0	0	+	+			
50b	—	—	—	—	0	(+)	(+)	0	0	+	+			
50c	—	—	—	—	0	(+)	(+)	0	0	+	+			
50d	—	—	—	—	0	(+)	(+)	0	0	+	+			
50e	—	—	—	—	0	(+)	(+)	0	0	+	+			
50f	—	—	—	—	0	(+)	(+)	0	0	+	+			
50g	—	—	—	—	0	(+)	(+)	0	0	+	+			
50h	—	—	—	—	0	(+)	(+)	0	0	+	+			
50i	—	—	—	—	0	(+)	(+)	0	0	+	+			
50j	—	—	—	—	0	(+)	(+)	0	0	+	+			
50k	—	—	—	—	0	(+)	(+)	0	0	+	+			
50l	—	—	—	—	0	(+)	(+)	0	0	+	+			
50m	—	—	—	—	0	(+)	(+)	0	0	+	+			
50n	—	—	—	—	0	(+)	(+)	0	0	+	+			
50o	—	—	—	—	0	(+)	(+)	0	0	+	+			
50p	—	—	—	—	0	(+)	(+)	0	0	+	+			
50q	—	—	—	—	0	(+)	(+)	0	0	+	+			
50r	—	—	—	—	0	(+)	(+)	0	0	+	+			
50s	—	—	—	—	0	(+)	(+)	0	0	+	+			
50t	—	—	—	—	0	(+)	(+)	0	0	+	+			
50u	—	—	—	—	0	(+)	(+)	0	0	+	+			
50v	—	—	—	—	0	(+)	(+)	0	0	+	+			
50w	—	—	—	—	0	(+)	(+)	0	0	+	+			
50x	—	—	—	—	0	(+)	(+)	0	0	+	+			
50y	—	—	—	—	0	(+)	(+)	0	0	+	+			
50z	—	—	—	—	0	(+)	(+)	0	0	+	+			
51a	—	—	—	—	0	(+)	(+)	0	0	+	+			
51b	—	—	—	—	0	(+)	(+)	0	0	+	+			
51c	—	—	—	—	0	(+)	(+)	0	0	+	+			
51d	—	—	—	—	0	(+)	(+)	0	0	+	+			
51e	—	—	—	—	0	(+)	(+)	0	0	+	+			
51f	—	—	—	—	0	(+)	(+)	0	0	+	+			
51g	—	—	—	—	0	(+)	(+)	0	0	+	+			
51h	—	—	—	—	0	(+)	(+)	0	0	+	+			
51i	—	—	—	—	0	(+)	(+)	0	0	+	+			
51j	—	—	—	—	0	(+)	(+)	0	0	+	+			
51k	—	—	—	—	0	(+)	(+)	0	0	+	+			
51l	—	—	—	—	0	(+)	(+)	0	0	+	+			
51m	—	—	—	—	0	(+)	(+)	0	0	+	+			
51n	—	—	—	—	0	(+)	(+)	0	0	+	+			
51o	—	—	—	—	0	(+)	(+)	0	0	+	+			
51p	—	—	—	—	0	(+)	(+)	0	0	+	+			
51q	—	—	—	—	0	(+)	(+)	0	0	+	+			
51r	—	—	—	—	0	(+)	(+)	0	0	+	+			
51s	—	—	—	—	0	(+)	(+)	0	0	+	+			
51t	—	—	—	—	0	(+)	(+)	0	0	+	+			
51u	—	—	—	—	0	(+)	(+)	0	0	+	+			
51v	—	—	—	—	0	(+)	(+)	0	0	+	+			
51w	—	—	—	—	0	(+)	(+)	0	0	+	+			
51x	—	—	—	—	0	(+)	(+)	0	0	+	+			
51y	—	—	—	—	0	(+)	(+)	0	0	+	+			
51z	—	—	—	—	0	(+)	(+)	0	0	+	+			
52a	—	—	—	—	0	(+)	(+)	0	0	+	+			
52b	—	—	—	—	0	(+)	(+)	0	0	+	+			
52c	—	—	—	—	0	(+)	(+)	0	0	+	+			
52d	—	—	—	—	0	(+)	(+)	0	0	+	+			
52e	—	—	—	—	0	(+)	(+)	0	0	+	+			
52f	—	—	—	—	0	(+)	(+)	0	0	+	+			
52g	—	—	—	—	0	(+)	(+)	0	0	+	+			
52h	—	—	—	—	0	(+)	(+)	0	0	+	+			
52i	—	—	—	—	0	(+)	(+)	0	0	+	+			
52j	—	—	—	—	0	(+)	(+)	0	0	+	+			
52k	—	—	—	—	0	(+)	(+)	0	0	+	+			
52l	—	—	—	—	0	(+)	(+)	0	0	+	+			
52m	—	—	—	—	0	(+)	(+)	0	0	+	+			
52n	—	—	—	—	0	(+)	(+)	0	0	+	+			
52o	—	—	—	—	0	(+)	(+)	0	0	+	+			
52p	—	—	—	—	0	(+)	(+)	0	0	+	+			
52q	—	—	—	—	0	(+)	(+)	0	0	+	+			
52r	—	—	—	—	0	(+)	(+)	0	0	+	+			
52s	—	—	—	—	0	(+)	(+)	0	0	+	+			
52t	—	—	—	—	0	(+)	(+)	0	0	+	+			
52u	—	—	—	—	0	(+)	(+)	0	0	+	+			
52v	—	—	—	—	0	(+)	(+)	0	0	+	+			
52w	—	—	—	—	0	(+)	(+)	0	0	+	+			
52x	—	—	—	—	0	(+)	(+)	0	0	+	+			
52y	—	—	—	—	0	(+)	(+)	0	0	+	+			
52z	—	—	—	—	0	(+)	(+)	0	0	+	+			
53a	—	—	—	—	0	(+)	(+)	0	0	+	+			
53b	—	—	—	—	0	(+)	(+)	0	0	+	+			
53c	—	—	—	—	0	(+)	(+)	0	0	+	+			
53d	—	—	—	—	0	(+)	(+)	0	0	+	+			
53e	—	—	—	—	0	(+)	(+)	0	0	+	+			
53f	—	—	—	—	0	(+)	(+)	0	0	+	+			
53g	—	—	—	—	0	(+)	(+)	0	0	+	+			
53h	—	—	—	—	0	(+)	(+)	0	0	+	+			
53i	—	—	—	—	0	(+)	(+)							

Table Xb (continued)

Case no	8				9		10			
	RPF (ml/min)				CPAH (ml/min)		CCr (ml/min)			
	R	L	Diff	/	R	L	R	L	Diff	
GROUP B1 Chronic pyelonephritis with signs of urinary tract obstruction (≥ 3 cardinal criteria)										
81	162	111	- 51	31	121	77	34	22	- 12	35
82a	—	—	—	—	70	79	14	15	+ 1	7
82b	—	—	—	—	—	—	—	—	—	—
82c	—	—	—	—	—	—	—	—	—	—
82d	—	—	—	—	—	—	—	—	—	—
83b	206	101	- 105	51	23	37	9	14	+ 5	16
84a	34	66	+ 32	48	22	50	8	19	+ 11	58
84b	—	—	—	—	—	—	—	—	—	—
85	—	—	—	—	—	—	—	—	—	—
86b	—	—	—	—	—	—	—	—	—	—
86c	—	—	—	—	—	—	—	—	—	—
87	—	—	—	—	—	—	—	—	—	—
88	—	—	—	—	13	57	11	34	+ 23	68
89	—	—	—	—	—	—	—	—	—	—
90c	—	—	—	—	—	—	—	—	—	—
90a	143	78	- 67	47	47	8	15	3	- 12	80
90b	—	—	—	—	—	—	—	—	—	—
91	—	—	—	—	—	—	—	—	—	—
92	—	—	—	—	—	—	—	—	—	—
93	—	—	—	—	—	—	—	—	—	—
GROUP B2 Renal disease (except pyelonephritis) with unilateral or mainly asymmetric involvement										
106	—	—	—	—	—	—	—	—	—	—
107	—	—	—	—	238	13	53	18	- 35	66
108	—	—	—	—	—	—	—	—	—	—
109	—	—	—	—	—	—	—	—	—	—
110	—	—	—	—	—	—	—	—	—	—
111	—	—	—	—	—	—	—	—	—	—
112	—	—	—	—	—	—	—	—	—	—
GROUP B3 Arterial hypertension with stenosis or aneurysm of the main renal artery										
117a	203	108	- 95	47	146	153	26	24	- 2	8
117b	—	—	—	—	166	193	10	13	+ 3	3
118	26	193	+ 167	87	31	159	9	33	+ 4	73
119	—	—	—	—	—	—	35	17	18	40
120	293	418	+ 125	30	245	341	29	41	- 12	29
121	—	—	—	—	143	151	31	35	- 4	11
122	—	—	—	—	—	—	35	40	15	27
123	273	184	- 89	33	239	26	30	40	0	40
124	—	—	—	—	—	—	70	49	21	10
125	—	—	—	—	—	—	—	—	—	—
126	—	—	—	—	—	—	26	46	- 20	41
127	181	167	14	8	146	141	34	27	7	21
128	—	—	—	—	173	61	14	30	16	43
129	—	—	—	—	—	—	—	—	—	—
GROUP B4 Renal disease with expected homogeneous involvement of both kidneys										
132	—	—	—	—	—	—	63	50	13	21
133	—	—	—	—	169	234	51	61	- 10	16
134	—	—	—	—	—	—	—	—	—	—
136	—	—	—	—	—	—	—	—	—	—
137	379	442	+ 63	14	315	387	45	62	17	7
138a	—	—	—	—	170	126	23	17	6	- 6
139	—	—	—	—	—	—	—	—	—	—
140	—	—	—	—	—	—	—	—	—	—
141	—	—	—	—	—	—	—	—	—	—
142	—	—	—	—	—	—	—	—	—	—
143	—	—	—	—	—	—	—	—	—	—
GROUP B5 Patients with symptoms from the lower urinary tract in whom involvement of the kidneys could not be ruled out										
148	—	—	—	—	—	—	—	—	—	—
149	—	—	—	—	—	—	—	—	—	—
150	—	—	—	—	—	—	—	—	—	—

Table Xb (continued)

Case no	11				12		13		14		15		16	
	Cia (ml/min)				Urine culture (bact/ml)		Roentgenol exam		Loin tenderness to palp		History of loin pain			
	R	L	Dff		R	L	R	L	R	L	R	L		
GROUP B1 Chronic pyelonephritis with signs of urinary tract obstruction (≥ 3 cardinal criteria)														
81	77	16	-11	41			(+)	+	0	0	-	-		
82a	15	12	-3	20	500 000	400 000	(+)	(+)	+	0	-	-		
82b	-	-	-	-	0	10 300	(+)	(+)	0	0	0	0		
83a	-	-	-	-	15 000	100 000	(+)	(+)	0	0	0	0		
83d	-	-	-	-		10 000	(+)	(+)	0	0	0	0		
83b	9	15	+6	46			(+)	(+)	0	(+)	0	0		
84a	7	15	+8	40	>100 000	50 000	+	(+)	0	0	0	0		
84b	-	-	-	-	0	0	+	(+)	0	0	0	0		
85	-	-	-	-	0	0	+	0	0	0	+	0		
Am chlor load														
ED *														
86a	-	-	-	-	4 700	3 500	(+)	+	0	0	0	0		
86c	-	-	-	-					0	0	0	0		
87	-	-	-	-	>100 000	>100 000	+	(+)	0	0	+	0		
88	-	-	-	-	0	00 000	(+)	+	0	0	+	0		
89	-	-	-	-	0	-	+	0	0	0	+	0		
90	-	-	-	-	0	30 000	0	+	0	0	+	0		
90a	15	3	12	80	5 000	150 000	(+)	+	0	0	+	+		
90b	-	-	-	-	>100 000	>100 000	(+)	+	0	0	+	+		
91	-	-	-	-	0	0	0	+	0	0	0	+		
92	-	-	-	-	0	70 000	(+)	+	0	0	0	+		
93	-	-	-	-	0	30 000	0	(+)	0	0	0	0		
GROUP B2 Renal disease (except pyelonephritis) with unilateral or mainly asymmetric involvement														
106	77	4	53	69	0	0	+	0	+	0	+	0		
107	-	-	-	-	0	0	0	0	0	0	0	0		
108	-	-	-	-	0	0	+	+	0	0	0	0		
109	-	-	-	-	0	0	+	+	0	0	0	0		
110	-	-	-	-	0	0	(+)	+	0	0	+	+		
111	-	-	-	-	0	0	0	0	0	0	0	0		
112	-	-	-	-	0	0	0	+	0	0	0	0		
Diuresis (ml/min)														
GROUP B3 Arterial hypertension with stenosis or aneurysm of the main renal artery														
117a	33	14	2	2			(+)	(+)	(+)	-			0.64	1.26
117b	4	1	4	33			0	0	0	0	-	-	0.30	0.36
118	1	46	45	68			0	0	0	0	-	-	0.06	1.10
119	-	-	-	-			0	0	0	0	-	-	3.3	0.87
120	37	44	7	16			(+)	0	0	0	-	-	0.47	0.96
121	79	6	3	10			0	0	0	0	-	-	-	-
122	-	-	-	-			0	0	0	0	-	-	-	-
123	-	-	-	-			0	0	0	0	-	-	0.64	0.43
124	-	-	-	-			0	0	0	0	-	-	0.65	3.93
125	-	-	-	-			0	0	0	0	-	-	1.03	1.06
126	-	-	-	-			0	0	0	0	-	-	-	-
127	34	34	0	0			0	+	0	0	-	-	0.5	0.73
128	-	-	-	-			0	0	0	0	-	-	0.5	0.79
129	-	-	-	-			0	0	0	0	-	-	3.07	1.53
GROUP B4 Renal disease with expected homogenous involvement of both kidneys														
133					0	0	0	0	0	0	-	-	2.11	2.16
135					0	0	0	0	0	0	-	-	0.95	0.9
134					0	0	0	0	0	0	-	-	-	-
136					0	0	0	0	0	0	-	-	-	-
137					0	0	0	0	0	0	-	-	-	-
138a	49	63	14	36			0	0	0	0	-	-	-	-
139	24	16	9	36			0	0	0	0	-	-	-	-
140					0	0	0	0	0	0	-	-	-	-
141							0	0	0	0	-	-	-	-
142							0	0	0	0	-	-	-	-
143							0	0	0	0	-	-	-	-
GROUP B5 Patients with symptoms from the lower urinary tract in whom involvement of the kidneys could not be ruled out														
148	-	-	-	-			0	0	0	0	0	0	-	-
149	-	-	-	-			0	0	0	0	0	0	-	-
150	-	-	-	-	0	0	0	0	(+)	+	0	0	-	-

Table Xb (continued)

Case no	2				3				4			
	Max conc ability (mOsmol/lit)				UNa (mEq/lit)				UK (mEq/lit)			
	R	L	Diff		R	L	Diff	/	R	L	Diff	
GROUP B1 Chronic pyelonephritis with signs of urinary tract obstruction (≥ 3 cardinal criteria)												
94	370	368	- 2	1	75	82	+ 7	9	-	-	-	-
95	493	461	- 32	6	-	-	-	-	-	-	-	-
96	635	933	+298	32	-	-	-	-	-	-	-	-
97	413	853	+440	44	91	177	+ 86	49	-	-	-	-
98	568	1075	+507	47	-	-	-	-	-	-	-	-
99a	525	480	- 45	9	-	-	-	-	-	-	-	-
99b	380	475	+ 95	20	84	-	- 1	1	-	-	-	-
100	740	1048	+298	28	-	-	-	-	-	-	-	-
101	245	1090	+755	76	110	230	+120	52	-	-	-	-
102a	441	436	- 5	1	-	-	-	-	-	-	-	-
102b	458	448	- 10	2	125	123	- 2	2	-	-	-	-
103	545	492	- 53	10	170	164	- 6	4	-	-	-	-
104	500	473	- 27	5	132	134	+ 2	1	-	-	-	-
105a	-	-	-	-	21	19	- 2	10	16	28	+12	43
105b	384	331	- 53	14	-	-	-	-	-	-	-	-
GROUP B2 Renal disease (except pyelonephritis) with unilateral or mainly asymmetric involvement												
113	796	488	-308	39	134	117	- 17	13	-	-	-	-
114	613	643	+ 30	5	170	180	+ 10	6	-	-	-	-
115	370	347	- 23	8	102	107	+ 5	0	-	-	-	-
116b	820	450	-370	45	-	-	-	-	-	-	-	-
GROUP B3 Arterial hypertension with stenosis or aneurysm of the main renal artery												
130	228	220	- 8	4	4	5	+ 1	20	2	2	0	0
131	-	-	-	-	24	25	+ 1	4	-	-	-	-
GROUP B4 Renal disease with expected homogeneous involvement of both kidneys												
135	515	570	+ 55	1	187	183	- 4	2	-	-	-	-
144	518	570	+ 52	2	-	-	-	-	-	-	-	-
145	298	296	- 2	1	90	100	+ 10	10	-	-	-	-
146	418	488	+ 70	12	105	108	+ 3	3	-	-	-	-
147	390	435	+ 45	10	74	69	- 5	7	-	-	-	-
GROUP B5 Patients with symptoms from the lower urinary tract in whom involvement of the kidneys could not be ruled out												
151	844	945	+ 101	10	240	230	- 10	4	60	62	2	1
152	928	945	+ 17	3	197	183	- 14	5	-	-	-	-

Table Xb (continued)

Case no	5			6			7		8		9		10	
	Uc (mEq/l)			U _{ptt}			Urine culture (bact/ml)		Roentgenol exam		Lo n tender ness to palp		H story of lom pain	
	R	L	Diff	R	L	Diff	R	L	R	L	R	L	R	L
GROUP B1 Chronic pyelonephritis with signs of urinary tract obstruction (≥ 3 cardinal criteria)														
94							32 m l	780 000	+	+	0	+	(+)	+
95							0	0	+	(+)	(+)	+	+	0
96							0	0	0	0	0	0	+	(+)
97							35 000	0	+	0	0	0	+	0
98							700 000	0	+	0	0	0	+	0
99a							0	160 000	(+)	+	0	0	0	0
99b	10	85	+15	18			100 000	900 000	(+)	+	0	0	0	0
100							0	300	+	0	0	0	0	0
101							>100 000	0	+	0	+	0	+	0
102a							0	0	+	0	0	0	0	0
102b							0	0	+	(+)	0	0	0	0
103	70	60	10	14			0	0	0	0	+	0	+	0
104	75	65	10	33			0	0	(+)	+	+	+	+	0
105a	10	35	+25	71			0	0	+	(+)	+	+	+	0
105b							9 000	0 000	+	(+)	0	0	0	0
GROUP B2 Renal disease (except pyelonephritis) with unilateral or nonlymphatic involvement														
113							0	80 000	0	+	0	0	0	0
114							5 000	0	+	+	+	+	+	+
115							0	70 000	+	+	0	+	0	+
116									+	0	0	+	0	+
GROUP B3 Arterial hypertension with stenosis or aneurysm of the renal artery														
111	18	15	0	0					+	(+)	0	0		
112	13	13	0	0					+	0	0	0		
GROUP B4 Renal disease with expected long-term survival of both kidneys														
111							0	700	0	0	0	0	0	0
114							0	0	(+)	(+)	0	0	0	0
115							0	0	(+)	(+)	0	0	(+)	0
116	80	75	5	6	76	68	0	0	0	0	0	0	0	0
117							0	0	+	+	0	0	0	0
GROUP B5 Patients with symptoms from the lower urinary tract in whom involvement of the kidneys could not be ruled out														
111							0	0	0	0	0	0	0	0
112							0	0	0	0	0	0	0	0

Table X1a Symmetry and asymmetry of individual renal functions and of other data

1	2	3	4	5	6	7	8	9	10	11	12	13
Case no	U_{osmol}	U_{Na}	U_K	U_{Cr}	U_{pH}	EP_{AH}	RPF	CCr	C_{In}	Urine culture	Roentge nol exam	Loin tender ness to palp
	$>10\%$	$\geq 10\%$	$\geq 10\%$	$>20\%$	≥ 0.05	$\geq 3\%$	$\geq 20\%$	$\geq 20\%$	$>20\%$	$\geq 10\%$		

GROUP A1 Chronic non obstructive pyelonephritis (≥ 3 cardinal criteria)

1	+	+	+	+	+	+	+	+	+	+	+	+
2a	+	+	+	+	+	+	+	+	+	+	+	+
2b	+	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+	+
4a	+	+	+	+	+	+	+	+	+	+	+	+
4b	+	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+	+
6b	+	+	+	+	+	+	+	+	+	+	+	+
6c	+	+	+	+	+	+	+	+	+	+	+	+
7a	+	+	+	+	+	+	+	+	+	+	+	+
7b	+	+	+	+	+	+	+	+	+	+	+	+
8a	+	+	+	+	+	+	+	+	+	+	+	+
8b	+	+	+	+	+	+	+	+	+	+	+	+
9a	+	+	+	+	+	+	+	+	+	+	+	+
9b	+	+	+	+	+	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+	+	+	+	+
11	+	+	+	+	+	+	+	+	+	+	+	+
12	+	+	+	+	+	+	+	+	+	+	+	+
13a	+	+	+	+	+	+	+	+	+	+	+	+
13b	+	+	+	+	+	+	+	+	+	+	+	+
14a	+	+	+	+	+	+	+	+	+	+	+	+
14b	+	+	+	+	+	+	+	+	+	+	+	+
14c	+	+	+	+	+	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+	+	+	+	+	+
16	+	+	+	+	+	+	+	+	+	+	+	+
17	+	+	+	+	+	+	+	+	+	+	+	+
18	+	+	+	+	+	+	+	+	+	+	+	+
19a	+	+	+	+	+	+	+	+	+	+	+	+
19b	+	+	+	+	+	+	+	+	+	+	+	+
20a	+	+	+	+	+	+	+	+	+	+	+	+
20b	+	+	+	+	+	+	+	+	+	+	+	+
21	+	+	+	+	+	+	+	+	+	+	+	+
22a	+	+	+	+	+	+	+	+	+	+	+	+
22b	+	+	+	+	+	+	+	+	+	+	+	+
23	+	+	+	+	+	+	+	+	+	+	+	+
24	+	+	+	+	+	+	+	+	+	+	+	+
25a	+	+	+	+	+	+	+	+	+	+	+	+
25b	+	+	+	+	+	+	+	+	+	+	+	+
26b	+	+	+	+	+	+	+	+	+	+	+	+
27	+	+	+	+	+	+	+	+	+	+	+	+
28c	+	+	+	+	+	+	+	+	+	+	+	+
29c	+	+	+	+	+	+	+	+	+	+	+	+
30b	+	+	+	+	+	+	+	+	+	+	+	+
31	+	+	+	+	+	+	+	+	+	+	+	+

GROUP A2 Acute and suspected chronic non-obstructive pyelonephritis (< 3 cardinal criteria)

70	+	+	+	+	+	+	+	+	+	+	+	+
71	+	+	+	+	+	+	+	+	+	+	+	+
72	+	+	+	+	+	+	+	+	+	+	+	+

Symbols

- Symmetry with reduced function
- [—] Symmetry with function within normal limits
- ↑ Asymmetry with poorer function of right kidney
- ↓ Asymmetry with poorer function of left kidney
- Not tested

The borderline of symmetry of the relevant function is given in each heading

Column 1

a, b c and d denote examinations on different occasions in the same patient

Table X1a (continued)

1	2	3	4	5	6	7	8	9	10
Case no	U _{Osmol} ≥10,	U _{Na} -10%	U _K ≥10%	U _{Cr} ≥20	U _{pH} ~0.05	Urine culture	Roentgenol exam	Loins tender-ness to palp	History of loins pain
GROUP A1 Chronic non-obstructive pyelonephritis (≥ 3 cardinal criteria)									
32									
33									
34									
35									
36									
37									
38									
39									
40									
41									
42									
43									
44									
45									
46									
47									
48									
49									
50									
51									
52									
53									
54									
55									
56									
57									
58									
59									
60									
61									
62									
63									
64									
65									
66									
67									
68									
69									
GROUP A2 Acute and suspected chronic non-obstructive pyelonephritis (< 3 cardinal criteria)									
73									
74									
75									
76									
77									
78									
79									
80									

Table Xib (continued)

1	2	3	4	5	6	7	8	9	10
Case no	U _{Osmol} ≥10 ³	U _{Na} ≥10 /	U _r ≥10 /	U _{Cr} ≥20 /	U _{pH} >0.05	Urine culture ≥10 ⁵ ×	Roentgenol exam.	Loir tenderness to palp	History of loir pain
GROUP B1 Chronic pyelonephritis with signs of urinary tract obstruction (≥ 3 cardinal criteria)									
94	+	+	+	+	+	+	+	+	+
95	+	+	+	+	+	+	+	+	+
96	+	+	+	+	+	+	+	+	+
97	+	+	+	+	+	+	+	+	+
98	+	+	+	+	+	+	+	+	+
99a	+	+	+	+	+	+	+	+	+
99b	+	+	+	+	+	+	+	+	+
100	+	+	+	+	+	+	+	+	+
101	+	+	+	+	+	+	+	+	+
102a	+	+	+	+	+	+	+	+	+
102b	+	+	+	+	+	+	+	+	+
103	+	+	+	+	+	+	+	+	+
104	+	+	+	+	+	+	+	+	+
105a	+	+	+	+	+	+	+	+	+
105b	+	+	+	+	+	+	+	+	+
GROUP B2 Renal disease (except pyelonephritis) with unilateral or mainly asymmetric involvement									
113	+	+	+	+	+	+	+	+	+
114	+	+	+	+	+	+	+	+	+
115	+	+	+	+	+	+	+	+	+
116b	+	+	+	+	+	+	+	+	+
GROUP B3 Arterial hypertension with stenosis or aneurysm of the main renal artery									
130	+	+	+	+	+	+	+	+	+
131	+	+	+	+	+	+	+	+	+
GROUP B4 Renal disease with expected homogeneous involvement of both kidneys									
135	+	+	+	+	+	+	+	+	+
144	+	+	+	+	+	+	+	+	+
145	+	+	+	+	+	+	+	+	+
146	+	+	+	+	+	+	+	+	+
147	+	+	+	+	+	+	+	+	+
GROUP B5 Patients with symptoms from the lower urinary tract in whom involvement of the kidneys could not be ruled out									
151	+	+	+	+	+	+	+	+	+
152	+	+	+	+	+	+	+	+	+

Table XII Incidence of symmetry and asymmetry of the individual renal functions and of other data in the different groups of diseases
When evaluating the GFR, only the C_{Cr} was taken into account and the C_{In} was omitted (cf p 69)

	U _{Osmol}							U _{Na}						
	A1	A2	B1	B2	B3	B4	B5	A1	A2	B1	B2	B3	B4	B5
Normal symmetry	1	4	—	—	—	1	1	—	—	—	—	—	—	—
Pathol symmetry	30	2	10	3	8	6	3	19	5	7	2	7	3	3
Consistent asymmetry	33	4	20	4	2	2	—	13	2	4	1	5	3	—
Inconsistent asymmetry	2	1	—	—	3	—	—	2	1	1	—	—	—	—
Indecisive	10	—	1	—	1	2	1	2	—	—	—	—	—	—
No of invest	76	11	31	7	14	11	5	36	8	12	3	14	6	3

	U _{Cr}							E _{PAH}						
	A1	A2	B1	B2	B3	B4	B5	A1	A2	B1	B2	B3	B4	B5
Normal symmetry	—	—	—	—	—	—	—	2	1	1	—	2	2	1
Pathol symmetry	15	2	5	—	2	1	—	4	—	1	—	2	2	—
Consistent asymmetry	10	2	2	—	2	—	—	16	2	9	3	5	2	1
Inconsistent asymmetry	—	—	1	1	—	—	—	3	—	—	1	1	2	—
Indecisive	—	—	—	—	—	—	—	7	—	1	3	2	3	1
No of invest	25	4	8	1	4	1	0	32	3	12	7	13	11	3

	RPF							C _{Cr}						
	A1	A2	B1	B2	B3	B4	B5	A1	A2	B1	B2	B3	B4	B5
Normal symmetry	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Pathol symmetry	5	—	—	—	1	1	—	2	1	1	—	2	1	—
Consistent asymmetry	1	—	3	—	4	—	—	6	—	4	1	9	3	—
Inconsistent asymmetry	—	—	—	—	—	—	—	1	—	—	—	—	—	—
Indecisive	1	—	1	—	—	—	—	1	—	1	—	1	—	—
No of invest	7	0	4	0	5	1	0	10	1	6	1	12	4	0

	Urine culture							Roentgenol exam						
	A1	A2	B1	B2	B3	B4	B5	A1	A2	B1	B2	B3	B4	B5
Normal symmetry	22	9	10	2	—	7	2	20	8	1	2	—	12	5
Pathol symmetry	16	—	8	—	—	—	—	14	—	5	2	2	3	—
Consistent asymmetry	15	—	11	3	—	—	—	38	3	22	6	8	—	—
Inconsistent asymmetry	3	—	—	—	—	—	—	1	—	1	—	3	—	—
Indecisive	4	1	1	—	—	—	—	8	—	5	1	2	—	—
No of invest	60	10	30	5	0	7	2	81	11	34	11	15	15	5

	Loim tenderness to palp							History of loim pain						
	A1	A2	B1	B2	B3	B4	B5	A1	A2	B1	B2	B3	B4	B5
Normal symmetry	51	11	22	6	12	16	4	26	5	11	3	—	9	4
Pathol symmetry	9	—	7	—	2	—	—	8	—	4	3	—	—	—
Consistent asymmetry	15	—	8	5	—	—	—	19	2	13	3	—	1	—
Inconsistent asymmetry	4	—	2	—	1	—	—	4	—	—	—	—	—	—
Indecisive	3	—	2	—	—	—	1	7	1	1	—	—	—	—
No of invest	82	11	35	11	15	16	5	64	8	29	9	0	10	4

CASE 17 The difference between the concentration ability of the kidneys was 10 per cent, the right kidney being poorer in this respect. On the pyelograms the outline of the left kidney was diffuse, the density of contrast medium was greatly reduced bilaterally but more on the left side. Table X shows that results of urine culture and of PAH extraction gave the same indications as the results of roentgenologic examination. The history indicated on the contrary that the right kidney had been involved more often and more severely.

In the foregoing case serving as an example there were signs that both kidneys were greatly damaged by chronic pyelonephritis and the differences in the various tests were generally small. This can explain why certain investigations gave contradictory information about which kidney was more involved. The results of determinations of the osmolality of the urine differed from those of other bilateral tests in one more patient in group A1 (case 40) and one patient in group A2 (case 76).

Determination of the renal PAH extraction showed divergent asymmetry in the following patients:

CASE 3 The left kidney showed poorer concentration ability as well as a lower urinary concentration of Na and creatinine. Moreover this kidney was smaller on the pyelograms (length of right kidney 14 cm, of left 17 cm) and had more pronounced anatomic changes than the right. The density of contrast medium was however about the same bilaterally. The PAH extraction by the right kidney was lower than that by the left whereas the Diodrast extraction was appreciably higher on the right side. It was evident from the history that there had been greater involvement of the right kidney.

CASE 79 The renal extraction of PAH was at variance with the roentgenologic features, history and findings at palpation. Thus the extraction by the left kidney was poorer whereas roentgenologic examination showed the right kidney to be smaller (length of right kidney 10.5 cm, of left 12 cm) and to have a thinner parenchyma. The history also gave the same indications with localization of tension and pain to the right loin, which was also tender to palpation.

CASE 138 The PAH extraction by the right kidney was greatly reduced (to 18.8 per cent) whereas that by the left was only slightly reduced (to 77.3 per cent). Two extraction determinations could be made on the right side but the lower value deviated from the mean by 27 per cent. This uncertain value is marked by an asterisk in Table X (*cf* Chap VIII). However the clearance of endogenous creatinine and of inulin showed poorer function of the left kidney. This was also borne out by determination of the urinary pH although the difference was extremely small (0.03). Neither determination of the osmolality of the urine, the history nor palpation over the loins provided evidence of any difference between the kidneys. The patient died just over a year after the investigation and autopsy also failed to establish any greater damage to either kidney. Thus both were of the same size and each weighed 100 g. Histologic examination showed signs of subchronic glomerulonephritis. — In this case the suspicion arises that some technical or other error might have been responsible for the great discrepancy in PAH extraction by the right kidney.

In 4 more cases (18, 106, 127 and 133) the extraction rate of PAH differed from those of other bilateral tests.

The results are summarized in Table XII and are discussed in Chapter X (pp 110—117).

CHAPTER X

GENERAL DISCUSSION

Evaluation of the present results particularly with respect to the existence of any asymmetry of renal function was based chiefly on the PAH extraction and the renal concentration ability. The reasons for this choice were

- 1 The tests are relatively easy to perform
- 2 The test of concentration ability does not require quantitative collection of the urine (164)
- 3 The tests provide information about the function of different parts of the nephron. This is because extraction (59) is dealt with mainly by the proximal convoluted tubules (cortex), and concentration by the loop of Henle and the collecting tubules (medulla), through the counter current mechanism.

Naturally, it would have been desirable to include the inulin and/or endogenous creatinine clearance as a measure of the glomerular filtration rate and the renal plasma flow as a measure of the renal blood flow. These determinations could however be made only on a limited scale and the same degree of reliability cannot be ascribed to the results as to those of the renal extraction and concentration tests.

A prerequisite for a clearance determination to be reliable is that the ureteric catheters are in position for a sufficiently long time to permit at least two (preferably three) clearance periods to be determined. In most of my cases, the renal concentration ability was determined on the same occasion

as the clearances so that the patient did not have to be catheterized twice at a short interval. The patient was therefore allowed no fluids for 24 hours before the test and was also given an injection of vasopressin (Pitressin®) on the previous evening. Consequently the urinary output was extremely small. This implies both that the clearance periods would have had to be comparatively long to decrease the error due to dead space and that even small losses of urine caused by leakage beside the catheter would have been a greater source of error than with ample diuresis.

For these reasons the glomerular filtration rate and renal plasma flow could not be estimated in some cases. In those cases in which the determinations were made the results cannot be regarded to be as reliable as determinations of the renal concentration ability and PAH extraction.

The other tests that were made were also judged to be of less consequence than the extraction and concentration abilities since they require further standardization of the conditions to be accorded full value. For example the pH of the urine is of limited value since no ammonium chloride load was used so that the ability to excrete hydrogen ions was not maximally utilized. Analogously the excretion of ammonia is difficult to assess since the glutaminase in the kidney and possibly other ammonia forming enzymes as well were not completely utilized.

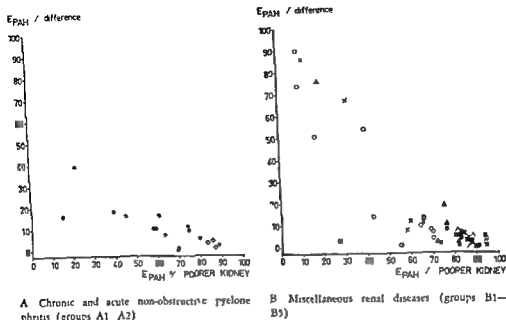


Fig 2 Difference (r_c) between the PAH extraction by the two kidneys (ordinate) and the extraction ratio (r_c) of the poorer kidney (abscissa) — For symbols see p 39

A Implications of Asymmetry of Individual Functions

In the majority of patients with chronic pyelonephritis there was measurable asymmetry of renal function. An account of the criteria of asymmetry applied in the present series is given in Chapter 1. It seemed appropriate to investigate the extent to which the degree of asymmetry was related to the degree of renal damage. It could be expected, *a priori*, to be more pronounced in chronic pyelonephritis with an increasing degree of renal damage since it seemed unlikely that the damage would involve both kidneys to the same extent.

Asymmetry of PAH extraction

The aforementioned presumption did in fact apply. However it seemed by no means

to be specific to pyelonephritis. Figure 2A shows the degree of asymmetry of PAH extraction in the cases of chronic and acute non-obstructive pyelonephritis (groups A1 A2) in relation to the extraction by the kidney that was poorer in this respect. The degree of asymmetry is given as the difference in extraction value between the kidneys expressed as a percentage of the extraction by the better one. This was considered to give a more correct idea of the asymmetry than the absolute difference. It is evident that if the extraction by one kidney was 30 per cent and that by the other 60 per cent the asymmetry is more marked than if the values were 65 and 95 per cent respectively. On the other hand it must be admitted that this method of calculation may be misleading if the values are greatly reduced. One can

scarcely speak of any noteworthy asymmetry if the extraction by one kidney is 10 and that by the other 20 per cent, whereas values of 30 and 60 per cent show a high degree of asymmetry.

In the present series, the former circumstance plays little role as far as PAH extraction is concerned, since few patients had such a severe decrease as to 10 per cent. However, with respect to renal concentration ability and the few determinations of the glomerular filtration rate, several patients had such low values that the percentage difference was extremely large, and might possibly give rise to misunderstanding, unless the mode of calculation is borne in mind.

The functional capacity of the poorer kidney was used as a measure of progression of the disease as regards the extraction ability. Obviously, in this connexion, no importance can be ascribed to the capacity of the better kidney since, theoretically, it can be only inappreciably involved irrespective of the degree of damage to the poorer kidney. For the same reason, the mean of the values for the two kidneys cannot be regarded as a measure of the extent of the pathologic process. It must, of course, be emphasized that the reduction in extraction ability alone was regarded as only one of many signs of the degree of severity of the disease.

It can be inferred from Figure 2B that the degree of asymmetry had a distinct tendency to increase with an increasing severity of renal damage. This can be interpreted to imply that when there is severe damage to the proximal tubules of the poorer kidney the two kidneys are usually involved to a different extent, whereas slight damage to these tubules is generally fairly symmetric. This tendency is evident when the differ-

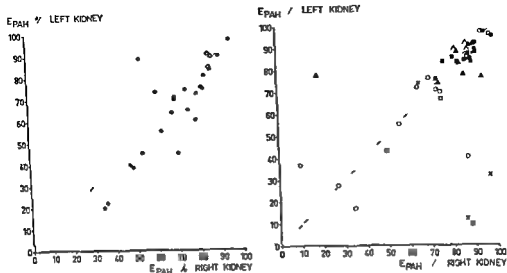
ences in E_{PAH} are plotted against the F_{PAH} of the poorer kidney, irrespective of whether these differences are expressed as percentages or numerically.

It is, however, apparent from Figure 2B that this relation also applied to groups B1—B5 (cases other than definite, chronic non-obstructive pyelonephritis). As far as obviously unilateral renal diseases are concerned, this was to be expected, but it was also found in a few patients in groups B4 and B5, in whom there was no reason to anticipate asymmetric function.

A noteworthy observation was made when studying which of the kidneys was more involved in the presence of asymmetry. Figure 3A shows that in most cases this was the left kidney. The mean value for extraction by the right kidney in the cases of chronic pyelonephritis was 71.12 ± 3.08 per cent ($n = 35$) and that by the left kidney 64.32 ± 3.54 per cent ($n = 35$). The difference 6.81 ± 2.07 per cent is highly significant ($p < 0.001$).

The implication of this finding is that in chronic pyelonephritis the proximal tubules of the left kidney are more susceptible to injury than the corresponding tubules of the right kidney.

The suspicion might arise that the lower extraction value was actually due to the renal vein blood obtained through the catheter in the left kidney being mixed with blood from the suprarenal and the testicular or ovarian vein (cf Chap VIII). This suspicion is, however, opposed by the fact that the PAH clearance was also lower in the left kidney in 7 of 10 cases and higher in only 3. Another argument indicating that the extraction values represent a real functional difference is that the catheter was advanced far into the renal vein and its



A Chronic and acute non obstructive pyelonephritis (groups A1-A7)

B Miscellaneous renal diseases (groups B1-B5)

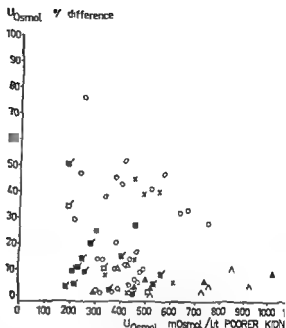
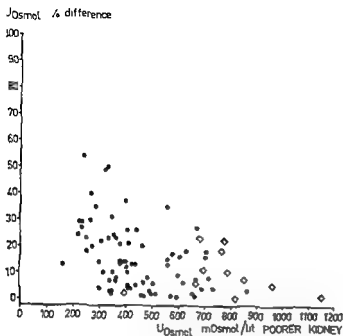
Fig 3 PAH extraction (%) by the left kidney (ordinate) and the right (abscissa) — The broken line represents symmetric extraction For symbols see p 39

position carefully checked at each examination. This diminished the risk of admixture with blood from the vena cava. The fact that the right renal vein is normally shorter than the left introduces another source of error. Thus when renal vein blood is withdrawn for determination of E_{PAH} there may be a greater risk of obtaining caval blood on the right side than on the left. However if this side difference was of any importance it would have resulted in apparently lower extraction on the right side. Consequently the difference noted cannot have been due to this source of error. Moreover the contribution of blood from the veins opening into the renal vein must have been inappreciable even in cases in which the renal plasma flow was low (Table VIII). Finally if the admixture of blood from tributaries had affected the extraction by the

left kidney this should have been noticeable in the determinations in healthy subjects as well which was not the case (Table III).

Bradley *et al* (25) also found that the left kidney generally had a lower PAH extraction than the right and ascribed this difference to the addition of blood from tributaries. These authors' case material consisted both of 22 subjects with healthy kidneys and of patients with such diseases as arterial hypertension and chronic glomerulonephritis. Their explanation does not however seem to be adequate (*cf* Chap VIII).

No corresponding dominance of poorer PAH extraction by the left kidney was observed in groups B1-B5 (Fig 3B). These groups are not, however, fully comparable since the great majority of patients had normal or only inappreciably reduced extraction. In two of the three cases in which



A Chronic and acute non obstructive pyelonephritis (groups A1 A2)

B Miscellaneous renal diseases (groups B1—B5)
— Symbols with a spike denote that the patient was not dehydrated and had not been given Pitressin®

Fig 4 Difference (%) between the concentration ability of the left and the right kidney (ordinate) and the concentration ability (mOsmol/lit) of the poorer kidney (abscissa) — For symbols see p 39

the greater decrease was noted for the left kidney the diagnosis was secondary chronic pyelonephritis and the third patient had hypoplasia of the left kidney. No significant difference was demonstrable between the right and left kidney in this respect.

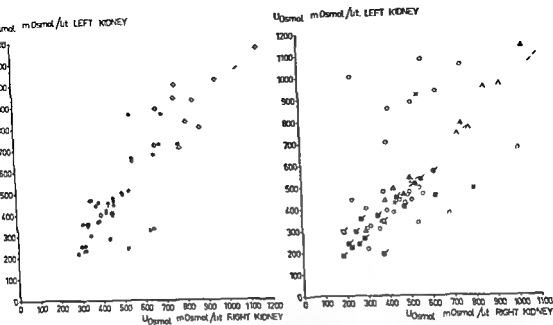
Asymmetry of concentration ability

It can be inferred from Figure 4A (groups A1, A2) that with respect to renal concentration ability as well, there was a tendency to increasing asymmetry with an increasing degree of renal damage. It must however be mentioned that some patients who had a good concentration ability in one kidney showed a fairly marked degree of asymmetry. Thus in these cases, the better kidney had completely or almost completely unim-

paired concentration ability. Similar observations were made in groups B1—B5 (Fig 4B). A few patients with renal damage that could be expected to be homogeneous (e.g. the nephrotic syndrome and chronic glomerulonephritis) showed only inappreciable asymmetry of concentration ability.

If a comparison is made between the concentration ability of the right and the left kidney (Fig 5A) the conditions differ distinctly from those in PAH extraction. Although the left kidney showed poorer concentration ability than the right in a larger number of cases (in 42 and 26 determinations respectively) the mean difference was relatively small and was not significant.

No tendency to a greater reduction in the concentration ability of the left kidney was



A Chronic and acute non-obstructive pyelonephritis (groups A1-A2)

B Miscellaneous renal diseases (groups B1-B5)
— Symbols with a spike denote that the patient was not dehydrated and had not been given Pitressin®

Fig. 5 Concentration ability (mOsmol/lit) of the left kidney (ordinate) and of the right (abscissa) — The broken line represents symmetric concentration ability. For symbols see p. 39

observed in groups B1-B5 (Fig. 5B). It is evident from this figure that remarkably many patients had a symmetric reduction in this function, as can also be seen in Figure 4B.

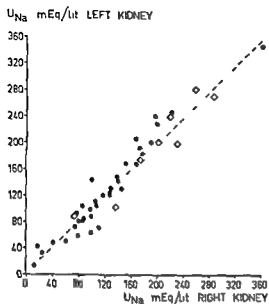
Asymmetry of sodium concentration

A comparison between the Na concentration in the urine from the right and the left kidney could have been expected to disclose approximately the same conditions as in the case of the osmolality. Figure 6A nevertheless shows that there was relatively little difference between the two kidneys in this respect. In the cases of pyelonephritis (groups A1-A2) the scattering around the

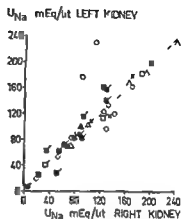
line of symmetry seemed in fact, even to be less than for the osmolality (Fig. 5A). The scattering was greater in groups B1-B5 (Fig. 6B) but it must be noted that the cases which showed the greatest deviation were precisely those in which asymmetry could be anticipated (e.g. two cases of chronic pyelonephritis with nephrolithiasis and several with unilateral stenosis of the main renal artery). The latter patients were not deprived of fluids before the test.

Asymmetry of endogenous creatinine, inulin and PAH clearances and of RPF

In the few cases in which the creatinine and/or inulin clearance by each of the kid-

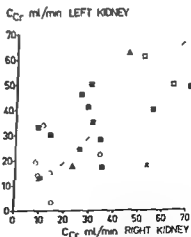
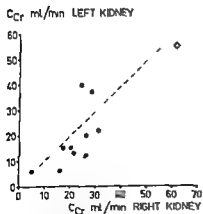


A Chronic and acute non obstructive pyelonephritis (groups A1 A2)



B Miscellaneous renal diseases (groups B1—B5)
— Symbols with a spike denote that the patient was not dehydrated and had not been given Pitressin®

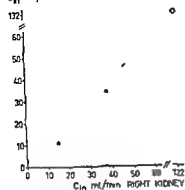
Fig 6 Sodium concentration (mEq/lit) in urine from the left kidney (ordinate) and from the right (abscissa) — The broken line represents symmetric sodium concentration For symbols see p 39



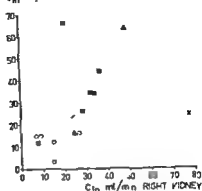
A Chronic and acute non obstructive pyelonephritis (groups A1 A2) B Miscellaneous renal diseases (groups B1—B5)

Fig 7 Endogenous creatinine clearance (ml/min) by the left kidney (ordinate) and by the right (abscissa) — The broken line represents symmetric clearance For symbols see p 39

C_{in} ml/min LEFT KIDNEY



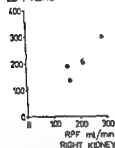
C_{in} ml/min LEFT KIDNEY



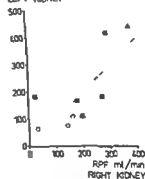
A Chronic and acute non-obstructive pyelonephritis (groups A1 A²) B Miscellaneous renal diseases (groups B1—B5)

Fig 8 Inulin clearance (ml/min) by the left kidney (ordinate) and by the right (abscissa) — The broken line represents symmetric clearance For symbols see p 39

RPF ml/min LEFT KIDNEY



RPF ml/min LEFT KIDNEY



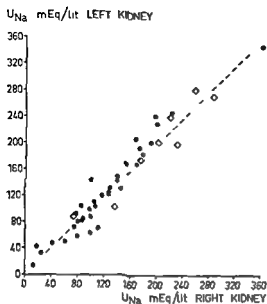
A Chronic and acute non-obstructive pyelonephritis (groups A1 A²) B Miscellaneous renal diseases (groups B1—B5)

Fig 9 Renal plasma flow (ml/min) in the left kidney (ordinate) and in the right (abscissa) — The broken line represents symmetric RPF For symbols see p 39

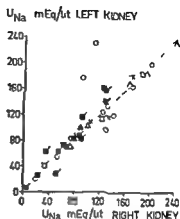
neys could be determined the value for the left kidney was lower in most of those in groups A1 and A2 (Figs 7A 8A). Obviously the PAH clearance in itself is of little value in cases such as these with greatly impaired renal function and decreased PAH extraction. The values have been included in

the tables only to illustrate the existence of asymmetry. However the difference is not significant so that no definite conclusions can be drawn. As previously mentioned the PAH clearance was lower on the left side in 7 of 10 cases in groups A1 and A2.

The RPF showed no preponderance of

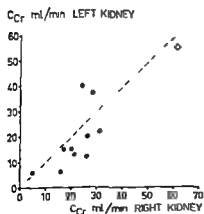


A Chronic and acute non-obstructive pyelonephritis (groups A1 A2)

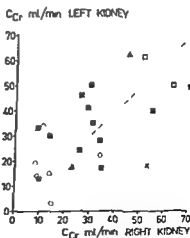


B Miscellaneous renal diseases (groups B1—B5)
— Symbols with a spike denote that the patient was not dehydrated and had not been given Pitressin®

Fig 6 Sodium concentration (mEq/lit) in urine from the left kidney (ordinate) and from the right (abscissa) — The broken line represents symmetric sodium concentration For symbols see p 39

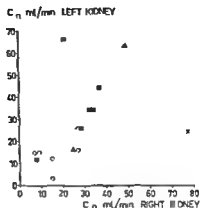
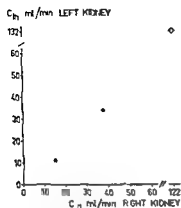


A Chronic and acute non-obstructive pyelonephritis (groups A1 A2)



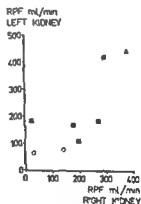
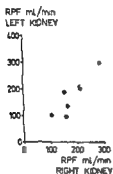
B Miscellaneous renal diseases (groups B1—B5)

Fig 7 Endogenous creatinine clearance (ml/min) by the left kidney (ordinate) and by the right (abscissa) — The broken line represents symmetric clearance For symbols see p 39



A Chronic and acute non-obstructive pyelonephritis (groups A1-A2) B Mellitus renal diseases (groups B1-B5)

Fig 8 Inulin clearance (ml/min) by the left kidney (ordinate) and by the right (abscissa) — The broken line represents symmetric clearance (Γ symbols see p 39)



A Chronic and acute nonobstructive pyelonephritis (group A1-A2) B Mellitus renal diseases (groups B1-B5)

Fig 9 Renal plasma flow (ml/min) in the left kidney (ordinate) and in the right (abscissa) — The broken line represents symmetric RPF (Γ symbols see p 39)

necks could be determined the value for the left kidney was lower in most of those in groups A1 and A2 (Figs 7A-8A). Obviously the PAH clearance in itself is of little value in cases such as these with greatly impaired renal function and decreased PAH excretion. The data have been included in

the tables only to illustrate the existence of asymmetry. However the difference is not significant so that no definite conclusions can be drawn. As previously mentioned the PAH clearance was lower on the left side in 7 of 10 cases in groups A1 and A2.

The RPF showed no preponderance of

either kidney in groups A1 and A2, on the contrary, these few values were surprisingly symmetric (Fig 9A)

The cases in groups B1—B5 were too heterogeneous to allow any uniform evaluation of the symmetry or asymmetry of the clearances in question, or of the RPF (Figs 7B, 8B, 9B) Moreover, the data obtained in groups B1—B5 were too few to permit any statistical analyses

Conclusions

Any speculations on why, in chronic pyelonephritis, the proximal tubules of the left kidney seemed to have been more involved than those of the right (difference between the PAH extraction on the two sides) must, for the time being, be limited to conjectures. The left kidney is, as a rule, slightly larger than the right (*cf* Chap II). The pedicle of the former is, however, longer than that of the latter, which might increase the risk of kinking of the ureter and vessels with urinary stasis or circulatory disturbances as a result. There is no reason to suspect that such kinking is more common in patients with chronic non obstructive pyelonephritis than in healthy subjects. On the other hand such an impediment could naturally accentuate the effect of existing pyelonephritis and in this way affect the PAH extraction among other functions. No other anatomic difference exists which might be responsible for a greater susceptibility of the left kidney to functional impairment.

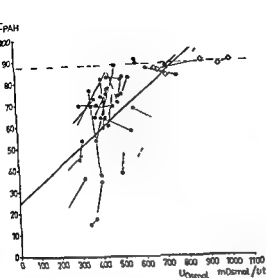
Another possible explanation is that the left kidney moves more than the right in connexion with respiration. This was demonstrated, *e.g.* by Hilgenfeldt (99) by means of pyelography. He stated that anatomists have found better fixation of the

left kidney than of the right to the diaphragm. This would allow it to follow the diaphragmatic excursions more closely. Hilgenfeldt gave a detailed anatomic description, and stated that the kidney's capsule — particularly the inner layers of fat — provide better sliding movements than the outer layers.

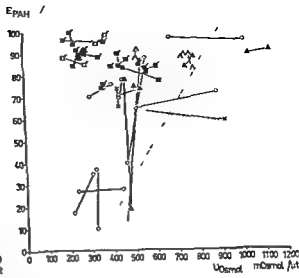
It seems plausible that this massage of the kidney favours the spreading of pyelonephritis, and perhaps also that the cortex is then involved to the greatest extent. This would explain why the excretion by the proximal tubules in particular and thereby the PAH extraction as well are affected to a greater extent in the left kidney. The same argument can be applied to the glomeruli. This hypothesis is not however supported by any other experimental evidence or objective observations than the aforementioned. Furthermore, Bacon (4) stated that he was unable to verify Hilgenfeldt's claim that the left kidney is more mobile than the right.

B Relation Between the Types of Functional Damage and Their Pathophysiologic Implications in the Individual Kidney

Comparisons between the type and degree of damage to the individual renal functions may afford a possibility of drawing conclusions about the topography of development and progression of a pathologic process in the renal tubules. If the extent of the process differs in the two kidneys examination of the bladder urine and of the extraction ratio in only one kidney may give misleading results.



A Chronic and acute non obstructive pyelonephritis (groups A1 A2) — The linear regression line for E_{PAH} values below 85% (—) follows the equation $y = 24.65 + 0.085x$ where y denotes E_{PAH} and x denotes U_{Osm} in dehydration ($n=45$). The equation for the regression line calculated on the cases with E_{PAH} above 85% (---) is not significant ($n=13$)
 cf p 99



B Miscellaneous renal diseases (groups B1—B5)
 — Symbols with a spike denote that the patient was not dehydrated and had not been given Pitressin®

Fig 10 PAH extraction ratio (% ordinate) and renal concentration ability (mOsmol/lit abscissa) — The values in the same patient are joined by a line. The broken line represents the hypothetical position of the values if the extraction ratio and concentration ability had been depressed to the same degree. For symbols see p 39

PAH extraction/concentration ability

Figure 10A shows the PAH extraction and the concentration ability of each kidney in relation to the contralateral one in the cases of chronic and acute non obstructive pyelonephritis (groups A1 A2). Each dot on the plot represents the PAH extraction by one kidney in relation to its concentration ability. The values for the right and left kidney in each case are joined by a line. Cases with the same value on the abscissa but a different one on the ordinate imply that the concentra-

tion ability is symmetric and the extraction asymmetric. Cases with the same value on the ordinate and a different one on the abscissa denote the reverse.

The fact that the dots for right and left kidney lie far from each other in most cases is already an indication that the functional impairment was usually asymmetric. In 8 cases the asymmetry was slight as regards PAH extraction and in at least 12 it was slight as regards concentration ability. Consequently the material does not permit the

either kidney in groups A1 and A2, on the contrary, these few values were surprisingly symmetric (Fig 9A)

The cases in groups B1—B5 were too heterogeneous to allow any uniform evaluation of the symmetry or asymmetry of the clearances in question, or of the RPF (Figs 7B, 8B, 9B). Moreover, the data obtained in groups B1—B5 were too few to permit any statistical analyses

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Another possible explanation is that the left kidney moves more than the right in connexion with respiration. This was demonstrated, *e.g.* by Hilgenfeldt (99) by means of pyelography. He stated that anatomists have found better fixation of the

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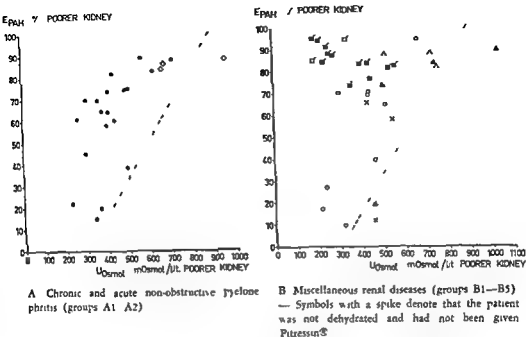


Fig 11 PAH extraction ratio (%) of the kidney with the lower E_{PAH} (ordinate) and concentration ability (mOsmol/lit) of the kidney with the lower U_{Osmol} (abscissa) The broken line represents the hypothetical position of the values if the extraction ratio and concentration ability had been depressed to the same degree — For symbols see p 39

distal tubules to the same degree the plotted values should lie along the aforementioned straight line irrespective of the grade of severity of the damage

However if a comparison is made between e.g. the extraction and the concentration ability in the present series it is found that the values do not follow this ideal line (Fig 10A). Without exception they lie on that side of the ideal line which implies higher extraction than concentration ability. This could be interpreted to mean that the extraction ability is relatively less reduced than the concentration ability. This in turn would imply that the injury has been greater to the medulla where concentration takes place than to the cortex where extrac-

tion takes place. This interpretation is compatible with the observation that in the cases with the lowest PAH extraction the concentration values approached the hypothetical origin. This would denote that in severely damaged kidneys there is an almost complete lack of both extraction and concentration.

The linear regression line for all the decreased E_{PAH} values (those below 85 per cent) in relation to U_{Osmol} followed the equation $y = 24.65 + 0.085x$ where y denotes E_{PAH} and x denotes U_{Osmol} in dehydration. The slope of the line is highly significant ($t = 3.73$, $n = 45$). The equation for the regression line calculated on the cases with E_{PAH} above 85 per cent is not

conclusion that certain parts of the tubules have a greater tendency than others to an asymmetric functional impairment

The results do, on the other hand, indicate that in chronic pyelonephritis the concentration ability is more severely and more often affected than is the extraction ability. It is evident from Figure 11A that several kidneys in the present material had exceedingly poor or almost lacking concentration ability, whereas the PAH extraction was only slightly reduced — in some cases it was even normal (It must be emphasized that the PAH concentration in the arterial blood was low in every case.) In no case did the reverse apply, *i.e.*, poor extraction with normal concentration ability.

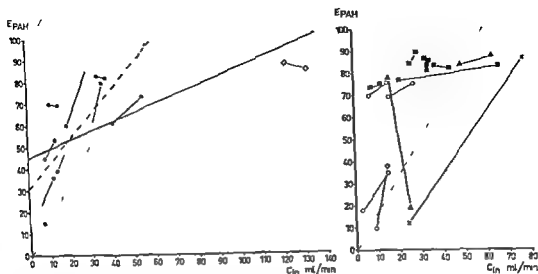
This observation might be explained in two ways. One is that the renal medulla (Henle's loop, the collecting ducts and the medullary circulation) is more severely damaged by the disease. The other is that it is more sensitive to the effects of the disease. Since it is probable that pyelonephritis frequently progresses in the retrograde direction, the former explanation appears more likely. Proof of this assumption requires comparative studies in other forms of renal disease. A small series of this kind is presented in Figure 10B but it is unfortunately too small and too heterogeneous to offer any convincing proof. Nevertheless in these few cases as well — some of which were secondary pyelonephritis — there was a lack of parallelism between the extraction and concentration abilities. Any definite conclusion must await a large number of cases.

It can be postulated that the PAH extraction is a measure of the functional capability of the proximal tubules (59) the concentration ability a measure of the function of the

collecting tubules, and the creatinine or inulin clearance a measure of the glomerular filtration. These parameters could then be used to compare the degree of damage to the different parts of the nephron. If they are plotted against each other in a coordinate system the origin should lie at approximately 300 mOsmol/lit (isostenuria) for the concentration ability, slightly above 0 for the extraction ability (due to the PAH filtered in the glomeruli) and at 0 for the glomerular filtration rate. If all parts of the nephron were invariably damaged to the same degree the values should lie on a line joining this origin with a point corresponding approximately to 85 per cent for the extraction ability, 800 mOsmol/lit for the concentration ability, and 30 ml/min for the glomerular filtration rate.

It is doubtful whether such a quantitative calculation of the degree of damage is justified. In the first place the normal maximum capacity of the individual patient's renal function cannot be established exactly. The foregoing values are therefore estimates based on the normal values given in the literature and the average age of the patients. Moreover it naturally cannot be claimed *e.g.* that the damage to the collecting tubules is twice as great when the concentration ability is 400 mOsmol/lit as when it is 300. Nor can it be stated that when the extraction by the proximal tubules is 30 per cent they are twice as much damaged as when the corresponding figure is 60 per cent. Thus the comparison was made with due reservation for the large sources of error inherent in the hypothesis. I nevertheless considered it to be of some interest for a study of the agreement or lack of agreement in the general relation between the tests.

If renal damage involves proximal and



A Chronic and acute non obstructive pyelonephritis (groups A1 A2) — The linear regression line for all values (—) follows the equation $y = 45.17 + 0.41x$ where y denotes E_{PAH} and x denotes C_i ($n = 20$) The linear regression line for all values except those in acute pyelonephritis (---) follows the equation $y = 30.95 + 1.13x$ ($n = 18$) cf p 103

B Miscellaneous renal diseases (groups B1—B5)

Fig. 1. Renal extraction of PAH (y ordinate) and inulin clearance (ml/min abscissa) — The values in the same patient are joined by a line. The broken line represents the hypothetical position of the values if the extraction rate and inulin clearance had been depressed to the same degree. For symbols see p 39

in the same direction for both these parameters in most cases of chronic and acute non obstructive pyelonephritis (Fig 13A). No definite correlation seemed on the other hand to be present between the size of the percentage differences for concentration ability *versus* Na concentration. In some cases the difference was even reversed i.e. the kidney which concentrated more effectively produced urine with a lower Na concentration than the other. Arterial hypertension (B.P. 180/110 mm Hg) was present in only one of these slightly deviating cases

(case 32). Renal angiography was not however performed in any of these patients.

The aforementioned discrepancy was still more conspicuous in the cases of other renal diseases (Fig 13B). It is striking to note that several cases of renal artery stenosis in particular showed this inverse relation. A similar observation has been reported earlier (e.g. 134).

It is difficult to find any explanation of the strange circumstance that the kidney with a poorer blood supply excretes urine containing more solutes and less sodium than that

significant ($t = 0.32$, $n = 13$). However, both these lines show a highly significant deviation from the hypothetical ideal line ($t = 4.50$ and 5.67 , respectively).

Returning to the few cases of other renal diseases, a tendency can, it is true, be seen to a displacement of the values to that side of the ideal line implying that concentration is relatively more impaired than extraction (Fig 10B). In cases with values in this position, it can be postulated that the damage has involved the collecting and the proximal tubules to approximately the same extent. It must, however, be emphasized that the series is far too small to permit any definite conclusions.

PAH extraction/inulin or endogenous creatinine clearance

When comparing the PAH extraction with the inulin or endogenous creatinine clearance the large sources of error that may be associated with unilateral clearance determinations must be borne in mind (*cf* Chap VIII). It was, however, found that reasons exist for believing that such errors were of relatively little consequence in the present series.

With reservation for these sources of error it is seen that the values for PAH extraction were also better than those for inulin clearance (Fig 12A). In the patients with pyelonephritis, the linear regression line for E_{PAH} in relation to C_{In} followed the equation $y = 45.17 + 0.41x$, where y denotes E_{PAH} and x denotes C_{In} . The slope of the line is significant ($t = 3.53$, $n = 20$), and shows a highly significant deviation from the hypothetical ideal line ($t = 10.50$). It is, however, highly questionable whether this regression line does, in fact represent the conditions in chronic pyelo-

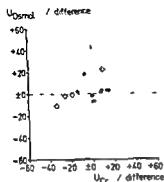
nephritis. This is because, if the only case of acute pyelonephritis in this plot is excluded, the regression line would have a completely different course (equation $y = 30.93 + 1.13x$). The slope of this line is then significant ($t = 4.20$, $n = 18$), but does not differ significantly from the hypothetical ideal line ($t = 2.04$, $n = 18$). If this represents the true conditions, it indicates that in chronic pyelonephritis the proximal tubules are generally less damaged than the glomeruli. It must, however, be pointed out that if the error due to leakage during collection of the urine takes effect in clearance determinations it would result precisely in such a shift of the values from the ideal line as that shown in Figure 12A.

My observation — confined to a few cases — is not in conformity with the results of Raaschou (200) and Kleeman *et al* (135) who found that the GFR was preserved to a greater extent than the function of the proximal tubules (T_{mD} , T_{mPAH}). Michie & Michie (163) demonstrated on the contrary that GFR and T_{mPAH} decreased proportionately in cases of chronic pyelonephritis.

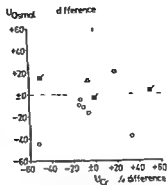
In the few cases of renal diseases other than chronic and acute non obstructive pyelonephritis there was a still greater tendency to retention of the extraction function even with greatly decreased GFR (Fig 12B). A comparison between PAH extraction and endogenous creatinine clearance gave similar results.

Concentration ability/Na concentration

The percentage difference between the kidneys with respect to the urinary concentration of Na and the corresponding difference in concentration ability were also compared. As could be expected there was a difference



A Chronic and acute non-obstructive pyelonephritis (groups A1-A2)



B Miscellaneous renal diseases (groups B1-B5)
— Symbols with a spike denote that the patient was not dehydrated and had not been given Pitressin®

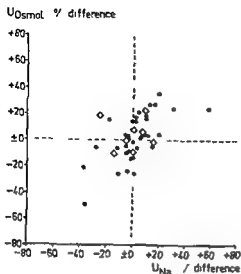
Fig. 14 Difference (%) between the concentration ability of the left and the right kidney (ordinate) and the corresponding difference (%) in urinary creatinine concentration (abscissa). — A plus sign before the numeral denotes that the value for the right kidney is higher than that for the left, and a minus sign *vice versa*. The broken lines represent symmetry. For symbols see p. 39

Concentration ability/inulin clearance

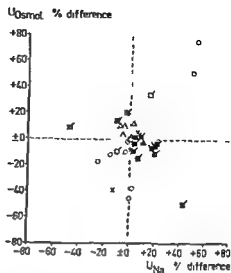
In a comparison between the concentration ability and inulin clearance it is found that all the patients in whom both tests were made had fairly or greatly reduced concentration ability, but that the glomerular filtration rate showed a wide range of variation (Fig. 15A). This indicates that Henle's loop and the collecting tubules are — or may be — more severely damaged than the glomeruli, whereas the reverse did not apply in any of my cases. The linear regression line for C_{in} in relation to U_{Osmol} followed the equation $y = 280.77 + 3.16x$ where y denotes U_{Osmol} in dehydration and x denotes C_{in} . The slope of the line is highly significant ($t = 7.46$, $n = 20$) as is its deviation from the hypothetical ideal line ($t = 16.19$).

This dissociation of function has been pointed out earlier by Brod (28, 29) and by Winberg (269) in acute pyelonephritis.

Brod stated that it is an extremely useful diagnostic sign in all developmental phases of pyelonephritis as well as for a differential diagnosis between chronic pyelonephritis and nephrosclerosis. In Figure 15B the osmolality of the urine is plotted against the inulin clearance in patients belonging to groups B1-B5, most of whom had essential hypertension. Here the values lay closer to the ideal line, and in most cases the functional impairment was parallel in both parameters in each of the kidneys. This is in agreement with Brod's results (29). In his series he found that the regression lines for chronic non-obstructive pyelonephritis, chronic glomerulonephritis and nephrosclerosis lay above each other in that order with the line for pyelonephritis closest to the x-axis (GFR). Bengtsson (10b) on the other hand noted no discrepancy between GFR and renal concentration ability in her series of chronic pyelonephritis. In another of her



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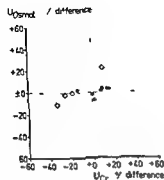
Fig 13 Difference (%) between the concentration ability of the left and the right kidney (ordinate) and the corresponding difference (%) in urinary sodium concentration (abscissa) — A plus sign before the numeral denotes that the value for the right kidney is higher than that for the left and a minus sign *vice versa*. The broken lines represent symmetry. For symbols see p 39

with a normal blood flow. Since it is known that the stenosed kidney also has a smaller urinary output (Howard's test) it is probable that the reabsorption of water as well is greater on this side (unless the glomerular filtration is considerably reduced). If this reabsorption is not due to the ADH but is more of the isotonic type, the concentration of urea and other osmotic substances would increase with decreasing Na concentration. It must however be pointed out that the osmolality of the urine was determined with out the patients being dehydrated, which diminishes the value of the comparison.

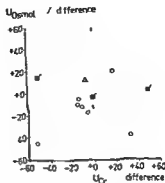
Concentration ability/urinary creatinine concentration

Since, in a dehydrated subject a difference between the concentration ability of the right

and left kidney is in all probability due to a difference in the reabsorption of water one can expect a corresponding difference in the urinary concentration of creatinine. Figure 14A shows the relation between asymmetry of concentration ability and the corresponding asymmetry of creatinine concentration. It can be seen that in the majority of cases the asymmetry of these two parameters of water reabsorption ran parallel. The range of the values was however too wide to warrant any attempt at calculating the regression or correlation. The reason for this relatively wide range is probably that the creatinine concentration is a function of the total reabsorption of water *i.e.* of the proximal tubules as well whereas the osmolality is not regulated until the urine reaches the collecting ducts.



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B Miscellaneous renal diseases (groups B1-B5)
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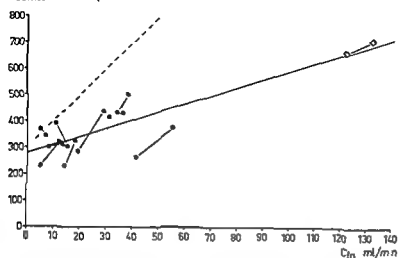
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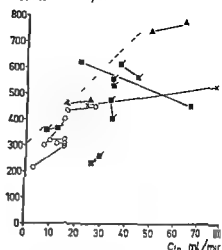
U_{Osmol} $mOsmol/lit$



A Chronic and acute non obstructive pyelonephritis (groups A1 A2) — The linear regression line for all values (—) follows the equation $y = 280.77 + 3.16x$ where y denotes U_{Osmol} in dehydration and x denotes C_{in} ($n=20$) cf p 103

Fig 15 Renal concentration ability ($mOsmol/lit$ ordinate) and inulin clearance (ml/min abscissa) — The values in the same patient are joined by a line The broken line represents the hypothetical position of the values if the concentration ability and inulin clearance had been depressed to the same degree For symbols see p 39

U_{Osmol} $mOsmol/lit$



B Miscellaneous renal diseases (groups B1—B5) — Symbols with a spike denote that the patient was not dehydrated and had not been given Pitressin®

groups — consisting of cases of chronic renal papillitis — the renal concentration ability was on the contrary more reduced than the GFR. She concluded that this classification of the material and exclusion of cases of acute pyelonephritis were responsible for the discrepancy between her latest results (10b) and her previous ones (10a) and between hers and those of Brod (29). Raaschou (200) also found that there was on the whole, a correlation between renal concentration ability and GFR (measured as the inulin and urea clearance).

A greater reduction in renal concentration ability than in GFR has been demonstrated in hydronephrosis — also without infection — in man (18, 269) as well as in acute obstruction of the urinary tract in dogs (114). Two of these authors made separate bilateral

studies of renal function (114, 269). My series contained only two patients with unilateral hydronephrosis (cases 109 and 110). Since the renal concentration test was not made in either of them no comparison can be made between the aforementioned functions.

Potassium deficiency (159, 207) like hypercalcaemia (74, 160) can also produce a greater reduction in renal concentration ability than in GFR. In these disorders the damage to the renal medulla seems to be caused by a similar mechanism (74). My series contained only one patient with potassium deficiency (case 19), but three with hypercalcaemia. In two of the latter hypercalcaemia was due to hyperparathyroidism (cases 105 and 109) and in the remaining one to renal tubular acidosis (case 115). The renal concen-

tration test was not made in case 109 (cf hydronephrosis). The other two patients with hypercalcaemia showed a greater reduction in concentration ability than in GFR which is in agreement with the aforementioned authors' observations.

Concentration ability, PAH extraction, endogenous creatinine clearance and RPF

Edvall (68) compared the EPAH and RPF in urological cases and found good agreement between them. In his series the effective RPF was determined as the PAH clearance in bladder urine whereas EPAH was determined in the urine from one kidney. In my material the RPF was plotted on one axis and the renal extraction ability and concentration ability respectively on the other. However, since no relation seemed to be present between these parameters, the graphic representation of the results has been omitted. On the other hand — as could be expected — a definite relation was present between RPF and endogenous creatinine clearance as demonstrated previously (135).

Conclusions

If any importance is to be ascribed to the comparisons presented in the foregoing as a quantitative measure of the relevant degree of damage, they imply that the concentrating part of the nephron (particularly Henle's loop and the collecting tubules) is most severely involved in chronic pyelonephritis. The glomeruli are next in order with respect to the degree of damage, whereas the proximal tubules seem as a rule to be less involved. In other words, the results indicate that in chronic pyelonephritis, the renal medulla is more severely damaged than the cortex.

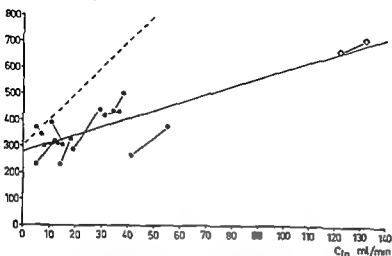
It is not until the renal damage has reached an extremely high degree — so that the function of all parts of the nephron is close to a minimum — that some parallelism is observed between the functional impairment of the various parts of the nephron, i.e. the values approach the origin on the plots. Obviously, in subjects with completely healthy kidneys, fairly good agreement would be present, since the peak of the ideal curve is plotted from the normal functional values. It is evident that there will be wide scattering of the values around this peak in view of the large variations which exist in renal concentration ability and glomerular filtration rate.

As stated earlier, the values in my patients belonging to group A2 (i.e. chiefly the cases of acute pyelonephritis) were included in the plots despite the uncertainty of the diagnosis. These results were concentrated around the upper part of the line which denotes a similar functional impairment — thus close to the values in healthy subjects. Most of these cases would then represent those in which renal damage was inappreciable.

The highest diastolic pressure of several recordings over 24 hours was plotted against the EPAH of the poorer kidney as well as against U_{Osmol} after withholding fluids. In groups A1 and A2, no correlation was demonstrable in either respect.

The results of the functional tests described in the foregoing are thus largely in agreement with the observations in animal experiments showing that the renal medulla is usually more sensitive to infection than the cortex in experimental chronic pyelonephritis (2, 80, 81, 89, 212, 213). In attempts to induce pyelonephritis in animals by local injection of bacteria at various sites

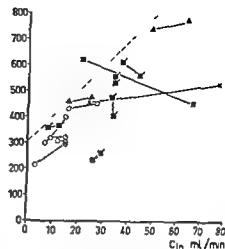
U_{Osmol} mOsmol/lit



A Chronic and acute non obstructive pyelonephritis (groups A1, A2) — The linear regression line for all values (—) follows the equation $y = 280.77 + 3.16x$ where y denotes U_{Osmol} in dehydration and x denotes C_{In} ($n=20$) cf § 103

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U_{Osmol} mOsmol/lit



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termining the nature of any renal damage. Their method was subsequently refined (15). It was based on the premise that a reduced extraction of PAH was to be ascribed essentially to two different causes: *i.e.*

1. Most PAH-excreting tubules were involved to some extent by the renal damage and

2. Only a limited number of tubules were damaged whereas others extracted PAH to the normal extent.

Smith and his co-workers (232, 234, 236, 237) had shown by means of so-called titration experiments that if the concentration of PAH in plasma was gradually raised from low to increasingly high levels, the tubular excretion of PAH initially rose in proportion to the tubular load of PAH (renal plasma flow \times plasma PAH concentration). However, when the plasma concentration exceeded a certain limit (saturation limit), the tubular excretion ceased to rise. It remained stable despite a rising plasma concentration. This implied that the tubules had reached their maximal excretory rate T_{mPAH} .

Josephson *et al.* (119) and Bergström *et al.* (15) tried to achieve corresponding results by determining the renal extraction of PAH instead of T_m , which is hard to determine. They reasoned that if the PAH concentration in plasma was gradually raised from low values, the renal extraction should remain unchanged as long as no tubule had reached its T_m . This implied in other words: for as long as the capacity of the excretory apparatus sufficed to deal with all the PAH delivered to it by the blood. The renal extraction should not start to fall until the concentration had reached T_m (the self-depression limit). Furthermore, this fall should run parallel to the rise in PAH concentration, since the kidneys would not be

able to extract from the blood more PAH than that represented by T_m plus the glomerular filtration. The saturation limit thus denotes the PAH concentration at which all the tubules had reached their maximal excretory capacity.

It was also presumed (119) that if some of the proximal tubules excreted normally, their self-depression limit should lie on a normal level (11–13 mg/100 ml plasma). If at a low PAH concentration the extraction was reduced because the damaged tubules no longer excreted, a rise in PAH concentration should not cause a further reduction in the total extraction until the maximal excretory capacity of the remaining normal tubules was reached — *i.e.* the self-depression limit should be normal. This would be ensured by the remaining healthy nephrons.

If on the other hand all proximal tubules were damaged — so that their excretory capacity was more generally reduced — decreased extraction should be observed even when the PAH concentration in plasma was inappreciably raised.

It can be pointed out that this determination does not require the urine to be measured or analyzed. In tests with a gradually rising PAH concentration in plasma (so-called staircase experiments), Josephson *et al.* (119) and Bergström *et al.* (15) did in fact in many cases note results which corresponded to their hypothesis.

The results were however often uncertain. This was partly because few cases of renal disease are actually so distinctly delimited that they give clear-cut results regarding the plasma concentration of PAH at which self-depression of extraction occurs. Moreover, it was difficult to administer the PAH infusion in such a way that the intended step

in the renal tissues Andriole & Epstein (2) also found the medulla to be more susceptible to the disease than the cortex On the basis of earlier experience, the authors ascribed this to the higher osmolality of the medullary tissues By giving the animals large quantities of water, they were able to keep the electrolyte concentration in the medulla on a low level, and thereby to decrease the incidence of pyelonephritis after infection Thus if pyelonephritis involves in the first place the renal medulla, the concentration ability should be more affected than the filtration and extraction abilities (74)

According to Beeson & Rowley (9) the chemical composition of the medullary tissues — particularly their content of ammonia and ammonia producing enzymes, which hamper complement activity — might also contribute to the greater vulnerability of this part of the kidney

The oldest explanation of a greater and earlier functional impairment of those parts of the nephron which are situated in the medulla is their anatomic localization in relation to the renal pelvis (55) This explanation appears highly probable at any rate in pyelonephritis caused by ascending infection

When comparing the occurrence of pathologic symptoms in the different parts of the nephron it is also necessary to bear in mind that the blood supply of the medulla is much smaller than that of the cortex It is considered that only a small percentage of the blood passing through the kidneys flows through the medulla whereas the greater part is received by the cortex (141 247 248)

Moreover, the oxygen supply of the medulla is also limited by the fact that oxygen is able to pass by diffusion from the descending to the ascending loop of the

arteriolar rectae, which implies that the renal papillae are susceptible to hypoxia (255) Ulfendahl (255) showed in addition in the same investigation that the oxygen tension is much higher in the renal cortex than in the medulla According to Trueta *et al* (252), the blood flow through the kidneys can be partly shunted between the cortex and the medullary tissue It has also been stated that the vascular system of the cortex and that of the medulla can act relatively independently (256) It is probable that these factors can combine to lower the resistance of the renal medulla to an infective agent

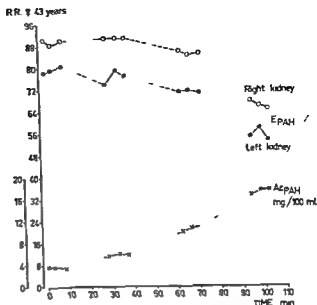
The present results show that in chronic non obstructive pyelonephritis in man as well, the function of the distal parts of the nephron is more depressed than that of the proximal ones However because of the way in which the disease develops, this does not apply in extremely slight cases nor in highly advanced ones

C Interpretation of a Decreased Extraction Ability

It has long been known that in persons with healthy kidneys about 90 per cent of the plasma PAH content is extracted during passage of the blood through the kidneys provided that the plasma PAH concentration is kept below the so called self depression limit (119) In healthy subjects this limit is considered to range from 11—13 mg/100 ml plasma If the concentration is raised above this limit the extraction starts to fall and continues to do so if the concentration continues to rise

Josephson *et al* (119) found in a study of the PAH accumulation in the kidneys of man that the value for the self depression limit might be of some importance for de

Fig 17 Extraction ratio (%) of PAH (o) and arterial PAH concentration (x) in a staircase experiment (case 92) — Iv infusion started 5 min before zero time. Infusion rate increased at 17, 40 and 74 min after zero time.



has therefore been used with advantage as a check on the PAH values. It must however be emphasized that the extraction of Diodrast is determined in whole blood whereas that of PAH is determined in plasma. This difference contributes to the discrepancies in extraction which may be observed.

The results of these determinations are best illustrated graphically. For reasons of space only two cases will be described.

CASE 3: a 41-year-old woman (Fig 16). Diagnosis: chronic pyelonephritis. She had a history of recurrent cystitis since childhood. Her blood pressure was normal as were the ocular fundi. The bladder urine contained relatively numerous WBC and a growth of *E. coli*. PAH clearance: 616 ml/min. Endogenous creatinine clearance: 93 ml/min. Inulin clearance: 115 ml/min. Serum creatinine: normal. Renal biopsy: acute to subacute interstitial nephritis. Pyelography showed that the right kidney was smaller than the left and had a greatly decreased density of intrast medium dysplasia was present in its lower pole. Determinations on catheter specimens

from the ureters disclosed better function of the left kidney with respect to concentration ability as well as Na and creatinine concentration (Table X).

It can be inferred from Figure 16 that when the PAH concentration in plasma rose above 9 mg/100 ml, there was no measurable effect on the PAH extraction in the left kidney and that of Diodrast fell only slightly whereas both extraction values fell markedly in the right kidney. At a low PAH concentration the left kidney showed normal extraction and the right an extremely slight reduction. It therefore seemed reasonable to presume the existence of slight focal damage to the right kidney whereas the left appeared to be undamaged.

CASE 9: a 43-year-old woman (Fig 17). Diagnosis: chronic pyelonephritis + renal papillary necrosis. During the past 6 years she had repeated attacks of cystitis accompanied by rigors and high fever. She also had attacks of renal colic with passage of concretions (renal papillae?). Her blood pressure was normal as were the ocular

G L 41 years

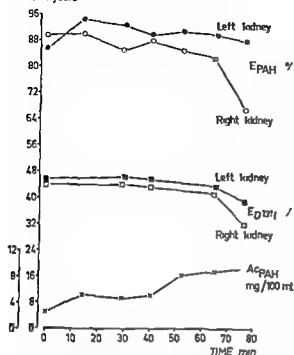


Fig 16 Extraction ratios (%) of PAH (o) and radioactive Diodrast ^{131}I (□) and arterial PAH concentration (v) in a staircase experiment (case 23) — I.v. infusion of PAH and Diodrast started 83 min before zero time. Infusion rate increased at 2 and 41 min after zero time

wise rise in plasma concentration was achieved. Obviously, if the whole kidney is involved by a pathologic process, a certain number of normally functioning nephrons usually remain. On the other hand one fraction of a majority of normal nephrons can be envisaged to be affected by its nearness to pathologic processes e.g. such in which toxic products are released. Furthermore PAH can — at any rate under certain conditions — be accumulated in the renal tissue (122). This may result in increased extraction as long as the accumulation is in progress (119) and a decreased extraction value on release of the accumulated PAH.

Another complicating factor is the risk of technical errors in the course of the determination. For example, the position of the

catheter tip may become altered during a long test, so that blood from the vena cava is mixed with that from the renal vein, or the patient may be affected e.g. by fatigue (cf Chap VIII).

Despite these limitations of the method I considered it of interest to test it in some cases in which the diagnosis of chronic pyelo-nephritis was regarded as unquestionable. The chief reasons for selecting patients precisely with chronic pyelonephritis were the often focal nature of the disease and the fact that it not infrequently involves only certain nephrons in a bundle (27). I made such staircase experiments in 14 cases. Since the object of the present investigation was to study the symmetry (or asymmetry) of renal function only brief mention will be made of some cases in which staircase experiments were made simultaneously on both kidneys.

In some of them a sufficiently high PAH concentration was never attained (which was not detected until the chemical analysis was made). In others, jumping extraction values were recorded. In five cases I succeeded in keeping a catheter in both renal veins during the rise in plasma PAH concentration so that the self depression limit of each of the kidneys could be studied simultaneously. Some patients were given radioactive Diodrast in the same infusion solution as PAH. This is because it has been shown that PAH and Diodrast are excreted by the same transport system or at any rate that they have some step in the transport system in common (cf Chap VII). If PAH and radioactive Diodrast are administered concurrently an increase in the plasma concentration of one of the substances has approximately the same depressive effect on the renal extraction of both substances (122). Radioactive Diodrast

RR 43 years

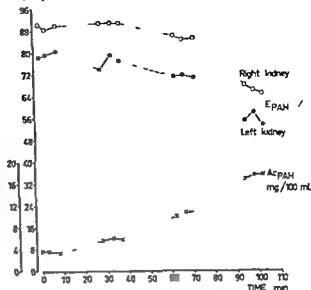


Fig 17 Extraction ratio (%) of PAH (o) and arterial PAH concentration (x) in a staircase experiment (case 9) — I.v infusion started 5 min before zero time. Infusion rate increased at 12.40 and 74 min after zero time

has therefore been used with advantage as a check on the PAH values. It must however be emphasized that the extraction of Diodrast is determined in whole blood whereas that of PAH is determined in plasma. This difference contributes to the discrepancies in extraction which may be observed.

The results of these determinations are best illustrated graphically. For reasons of space only two cases will be described.

CASE 73 a 41 year old woman (Fig 16). **Diagnosis:** chronic pyelonephritis. She had a history of recurrent cystitis since childhood. Her blood pressure was normal as were the ocular fundi. The bladder urine contained relatively numerous WBC and a growth of *E. coli*. PAH clearance 616 ml/min. Endogenous creatinine clearance 93 ml/min. Inulin clearance 115 ml/min. Serum creatinine normal. Renal biopsy acute + subacute interstitial nephritis. Pyelography showed that the right kidney was smaller than the left and had a greatly decreased density of contrast medium. Dysplasia was present in its lower pole. Determinations on catheter specimens

from the ureters disclosed better function of the left kidney with respect to concentration ability as well as \dot{V}_a and creatinine concentration (Table X).

It can be inferred from Figure 16 that when the PAH concentration in plasma rose above 9 mg/100 ml, there was no measurable effect on the PAH extraction in the left kidney and that of Diodrast fell only slightly whereas both extraction values fell markedly in the right kidney. At a low PAH concentration the left kidney showed normal extraction and the right an extremely slight reduction. It therefore seemed reasonable to presume the existence of slight focal damage to the right kidney whereas the left appeared to be undamaged.

CASE 9* a 43 year old woman (Fig 17). **Diagnosis:** chronic pyelonephritis + renal papillary necrosis. During the past 11 years she had repeated attacks of cystitis accompanied by rigors and high fever. She also had attacks of renal colic with passage of concretions (renal papillae?). Her blood pressure was normal as were the ocular

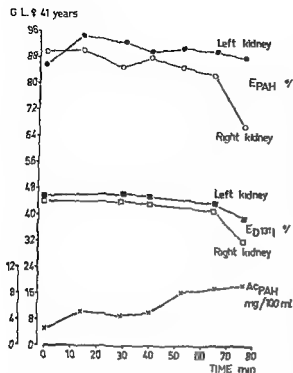


Fig. 16 Extraction ratios (%) of PAH (o) and radioactive Diodrast 131I (□) and arterial PAH concentration (x) in a staircase experiment (case 23) — 1% infusion of PAH and Diodrast started 83 min before zero time. Infusion rate increased at 2 and 41 min after zero time

wise rise in plasma concentration was achieved. Obviously if the whole kidney is involved by a pathologic process, a certain number of normally functioning nephrons usually remain. On the other hand one fraction of a majority of normal nephrons can be envisaged to be affected by its nearness to pathologic processes e.g. such in which toxic products are released; Furthermore, PAH can — at any rate under certain conditions — be accumulated in the renal tissue (122). This may result in increased extraction as long as the accumulation is in progress (119) and a decreased extraction value on release of the accumulated PAH.

Another complicating factor is the risk of technical errors in the course of the determination. For example the position of the

catheter tip may become altered during a long test, so that blood from the vena cava is mixed with that from the renal vein, or the patient may be affected, e.g. by fatigue (cf Chap VIII).

Despite these limitations of the method I considered it of interest to test it in some cases in which the diagnosis of chronic pyelonephritis was regarded as unquestionable. The chief reasons for selecting patients precisely with chronic pyelonephritis were the often focal nature of the disease, and the fact that it not infrequently involves only certain nephrons in a bundle (27). I made such staircase experiments in 14 cases. Since the object of the present investigation was to study the symmetry (or asymmetry) of renal function only brief mention will be made of some cases in which staircase experiments were made simultaneously on both kidneys.

In some of them a sufficiently high PAH concentration was never attained (which was not detected until the chemical analysis was made). In others jumping extraction values were recorded. In five cases, I succeeded in keeping a catheter in both renal veins during the rise in plasma PAH concentration so that the self depression limit of each of the kidneys could be studied simultaneously. Some patients were given radioactive Diodrast in the same infusion solution as PAH. This is because it has been shown that PAH and Diodrast are excreted by the same transport system or at any rate that they have some step in the transport system in common (cf Chap VII). If PAH and radioactive Diodrast are administered concurrently an increase in the plasma concentration of one of the substances has approximately the same depressive effect on the renal extraction of both substances (122). Radioactive Diodrast

Other split function tests such as $T_m\text{PAH}$ T_m glucose product of ammonia and renal acidifying ability require loading arrangements that are difficult or impossible to achieve in each kidney separately if the aforementioned functions are to be studied concurrently.

The asymmetry of the arteriovenous oxygen difference has also been studied in various clinical conditions (181).

As indicated by Tables X, XI and XII most renal functions proved to be asymmetric in those cases where asymmetry was expected insofar as any conclusions can be drawn from the scanty data in some of the groups. It is also seen that in every group there were certain cases in which symmetry between the kidneys was not demonstrated for some function thus applied to all the parameters tested. In some cases it was difficult to decide whether asymmetry did in fact exist since the right kidney preponderated in one respect and the left in another. Divergent asymmetry of this kind was relatively common as regards the results of PAH extraction in patients with chronic nonobstructive pyelonephritis. Divergent asymmetry was also found in a few cases with respect to tenderness to palpation, U_{Na} and anamnestic data. A noteworthy feature is that most of these cases belonged to groups A1, A2 and B1 (chronic and acute nonobstructive and obstructive pyelonephritis). A possible explanation of the divergence is that this disease can always be regarded as bilateral; consequently one or both of the kidneys will dominate for different functions and symptoms at different times. Divergent asymmetry was rare in the case of roentgenologic examination, U_{Osm} , U_{Cr} , RPF and GFR.

Symmetric renal function was indicated by some tests in all groups. The endogenous

creatinine clearance indicated divergent asymmetry only in one case in group A1. Since the creatinine clearance was determined in more cases than the inulin clearance, evaluation of asymmetry of GFR was based on the former determination.

It cannot be concluded from the present study that the presence of asymmetry is a sufficient reason for making a particular diagnosis. It can, however, be stated that asymmetry with respect to concentration ability and U_{Cr} was more common in the cases of pyelonephritis (groups A1, A2 and B1) than in the other groups, but that no such difference could be demonstrated for PAH extraction, roentgenologic examination, U_{Na} or GFR.

Occurrence of Asymmetric Function in Certain Renal Diseases

Chronic and acute pyelonephritis (groups A1, A2 and B1)

Asymmetric renal function with respect to a number of parameters has previously been reported in cases of acute and chronic pyelonephritis. Some of these references are listed in Table II.

In chronic pyelonephritis it must always be assumed that both kidneys are involved; this has been confirmed by e.g. Brod (28), Raaschou (200) and Sensenbach (229). All seven cases of chronic pyelonephritis in the present series in which autopsy was performed had contracted kidneys (cases 5, 19, 28, 35, 39, 83, 90). No substantial difference could be detected between the two kidneys with respect to weight and size, presumably because they were in an advanced stage of anatomic destruction. The same trend applied to function. Irrespective of the aetiology, some of the patients with chronic pyelonephritis had advanced renal insufficiency.

fundi PAH clearance 509 ml/min Endogenous creatinine clearance 81 ml/min The serum creatinine calcium and phosphate phosphorus concentrations were normal Renal biopsy focal interstitial inflammation as in pyelonephritis Pyelography showed that both kidneys were of the same size but that the parenchyma of the left was reduced and all its papillae had been sloughed Similar but less marked lesions were present in the right kidney Determinations on catheter specimens showed a moderate number of WBC and RBC as well as a growth of *E. coli* (70 000/ml) in the urine from the left ureter but not in that from the right With respect to concentration ability and pH of the urine as well as creatinine concentration the left kidney showed poorer function than the right (Table X)

Figure 17 shows normal extraction by the right kidney with a self depression limit of about 11 mg/100 ml The left kidney had a lower extraction ratio irrespective of the PAH concentration The self depression limit was identical to that on the right side In this case, the results suggest focal damage to the left kidney

The results in these two cases illustrate that the staircase method may in some cases give additional information about the type and extent of involvement of the kidneys Despite some uncertainty the method may be of value for estimating the state of the excretory apparatus i.e. the proximal tubules This applies especially in a study of the possible asymmetry of function of this apparatus Obviously the method is too circumstantial and the procedure too difficult for it to have any routine clinical applicability

D Clinical Value of Testing for Asymmetry of Renal Function

Catheterization of the ureters or renal veins does not involve much discomfort for the patient and neither measure is risky or

difficult to perform for anyone with some experience Both procedures are, however somewhat lengthy, particularly when combined with a functional diagnosis of the type described here Moreover, they require the assistance of trained staff special premises and — at any rate as far as renal vein catheterization is concerned — expensive equipment It is beyond the scope of the present study to attempt an assessment of the clinical value of ureteric and renal vein catheterization in general It nevertheless seems motivated to try to estimate the value — and in particular the clinical advantages — of ascertaining the presence of any asymmetry of renal function with these procedures above all in cases in which a definite diagnosis cannot be made with simpler methods Since chronic pyelonephritis is the disease (or group of diseases) constituting the majority of the cases in the present study the following discussion is primarily related to this complaint although other forms of renal diseases are also considered

Methods for Studies of Each Kidney Separately

As previously stated, the clearance of inulin and endogenous creatinine has been used to assess the glomerular filtration rate the PAH extraction to assess the function of the proximal tubules and the renal concentration ability to express the function in the distal parts of the nephron The creatinine concentration in the urine has been taken as an approximate measure of water reabsorption

In the present study the PAH extraction and the concentration ability were mainly used as parameters of renal function for the reasons given on page 88

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concretion had formed in the right kidney possibly in connexion with the bacteriuria. At the first examination, the concentration ability of the right kidney was 9 per cent better than that of the left. On the second occasion this ability was unchanged in the left kidney but had deteriorated considerably in the right (in which the concretion was present) and the difference between the two was then 7 per cent with the better value for the left kidney.

For practical reasons cases of chronic pyelonephritis can be divided into two categories according to the difficulty involved in establishing the diagnosis.

One category consists of cases in which the diagnosis is fairly obvious chiefly because they fulfil the cardinal criteria discussed in Chapter III. The other category consists of cases in which a diagnosis of chronic pyelonephritis is probable but not definite.

Among the cases in the first category are those for instance in which the history indicates repeated attacks of pyelonephritis, the urine contains bacteria and other elements suggestive of pyelonephritis and the roentgenologic features are characteristic (e.g. deformation of calyces, difference between the size of the kidneys and scarred indentations on the kidney surface).

In cases in which fulfilment of the cardinal criteria makes a diagnosis of chronic pyelonephritis fairly obvious it may often seem superfluous to test for asymmetry of renal function. Nevertheless it may be motivated to study the two kidneys separately to determine for instance whether bacteriuria originates from only one of them or whether function is depressed in both although a concretion has been detected in one kidney only. Such a study is particularly valuable when surgery is being considered. This is illustrated by the following case.

CASE 97 a 37 year old woman. Diagnosis: chronic pyelonephritis + right sided nephrolithiasis + arterial hypertension + obesity. About 2 years before the current course of treatment arterial hypertension and proteinuria had been detected in connexion with pregnancy.

The 24 hour blood pressure ranged from 180/120 to 140/100 mm Hg (mean 160/110 mm). Ocular fundi changes corresponding to FH I. Roentgenologic examination of the heart size and configuration normal. Serum creatinine normal. PAH and endogenous creatinine clearances in bladder urine 39" and 101 ml/min respectively. Excretory pyelography showed kidneys of equal size (R 15 cm long L 16 cm) with large coral concretion in the right kidney. Selective studies of renal function indicated that the right kidney's concentration ability and the Na concentration in its urine were substantially lower than the left kidney's. 33 000 bacteria/ml were found in the right kidney's urine but none in the left. PAH excretion was not tested. Right sided nephrectomy was performed. Although the patient's urine has been free from bacteria at several check ups her blood pressure has not fallen appreciably. Histologic examination of the right kidney showed the changes associated with chronic pyelonephritis.

The other category of cases — in which the diagnosis of chronic pyelonephritis is probable but not definite — consists of patients presenting symptoms and signs which are either obscured by some other renal disease or which do not satisfy the cardinal criteria. Patients in the aforementioned first category need not invariably be submitted to the extensive questioning and investigations required to test these criteria. In the present category on the other hand these criteria are of great value since the diagnosis can be regarded as comparatively definite if at least three of them are complied with. Nevertheless useful information can be obtained by making other investigations (e.g. selective studies of renal function). The factor that has proved to be of paramount value for strengthening the diagnosis has

as a result of pronounced anatomic changes, which were established roentgenologically (cases 35 and 39) In these cases, it was difficult to detect any functional pattern in the impairment of different renal functions This circumstance which has already been discussed in Chapter V, is in agreement with reports by e.g. Bengtsson (10b) and Klee man *et al* (135)

The results in question nevertheless show that a bilateral study of renal function can be an aid in diagnosis, especially in a number of cases of chronic pyelonephritis in which the diagnosis was either supported by the bilateral study or would have been impossible to make without it As a rule, these patients fulfilled only a few cardinal diagnostic criteria (cases 21, 27, 33, 43, 52, 91, 96)

As already mentioned, repeated determinations of renal function were made in a number of patients undergoing long term anti bacterial therapy This was done in an attempt to obtain an objective measure of the value of this form of therapy In 13 of the 16 cases, the function of at least one kidney improved during therapy (cases 2, 4a, b, 6, 7, 9, 13, 14, 19, 20, 25, 82, 86, 90 Table X)

CASE 14 a 26 year old woman Ureteric and renal vein catheterization were performed twice (case 14 a and b) at an interval of 6 weeks and a third time (14 c) 13 months later On the first occasion the PAH extraction was strongly depressed on both sides particularly the left The concentration ability was also reduced by about the same amount on both sides On the third occasion considerable subjective improvement was noted In addition the endogenous creatinine clearance had more than doubled and the urine was free from bacteria Moreover both concentration ability and PAH extraction had improved on both sides The interesting feature was that — parallel to this general improvement — the differ

ence between the extraction by the two kidneys was much less (7 instead of 22 per cent) On the other hand the corresponding difference with respect to concentration ability had increased the left kidney then excreting the more concentrated urine

These findings can be interpreted as bilateral improvement, in which the left kidney — particularly its concentrating apparatus — had made the better recovery

Renal function deteriorated in three patients during the interval between examinations

CASE 4 a 45 year old woman Definite improvement was observed between the first two examinations (4 a and b group A1) but later on the condition of the patient was influenced by the formation of concretions in the left renal pelvis (4 c group B1) probably owing to a recurrence of the urinary tract infection As a result the PAH extraction by the left kidney was considerably reduced but this was paralleled by an improvement in the concentration ability of both kidneys Here we have the paradoxical phenomenon of cortical function (proximal tubules) deteriorating on the affected side together with an apparent improvement in medullary function (collecting tubules and the loop of Henle)

CASE 84 a 28 year old woman She also had a recurrence of bacteriuria at the time of the second examination which may explain the reduction in PAH extraction At the first examination the PAH extraction by the two kidneys differed by 7 per cent the corresponding difference for the concentration ability being 25 per cent The latter function was not determined on the second occasion when the difference in PAH extraction had diminished to only 1 per cent On both occasions the patient had bilateral renal concretions and calcareous papillae although these regressed considerably during the observation period

CASE 99 a 45 year old woman also had a recurrence of the urinary tract infection (*Proteus mirabilis*) at the time of the second examination 2 years after the first In the interval a small

In the 10 cases of unilateral stenosis of the main renal artery in which the PAH extraction could be determined it was found to be on a satisfactory level with little difference between the kidneys. In 3 of these cases extraction by the stenosed kidney was slightly poorer, in 4 it was slightly better, whereas in 3 there was no difference between the kidneys. I have been unable to trace any study in the literature in which the PAH extraction was determined bilaterally in patients with this disease.

In contrast to the procedure in chronic pyelonephritis and in the other groups in this study, bilateral ureteric catheterization in cases of hypertension and disease of the main renal artery was performed with the patient in a state of moderate hydration. This was done to enable detection of any difference between the urinary flow from the two sides. Forced hydration has been suggested as a mean of accentuating this difference, e.g. by Hulet *et al* (109), Kjellbo *et al* (134) and Stames *et al* (241). Naturally this precludes concurrent determination of the concentration ability. A comparison between the osmolality of the urine from either kidney can nevertheless be worth while in patients with unilateral stenosis of the main renal artery, despite the fact that the result often varies with time if the patient is hydrated. However in 2 of the 10 cases in which this determination was made the osmolality was higher on the side with the stenosed artery, lower in 2 cases and the same on both sides in six. Higher osmolality of the urine from the ischaemic kidney has been reported previously (64, 65, 134, 220).

Howard's test (106) — which is based on White's (269) observations in experimental constriction of one of the main renal

arteries in the dog (a 50 per cent reduction in urinary flow and at least a 15 per cent lower Na concentration in the urine from the ischaemic kidney) — has been an important tool in evaluation of stenosis of the main renal artery ever since its introduction in 1953 (5, 34, 56, 57, 210, 241, 276). In the present study the findings with respect to the urinary flow deviated from the stipulated pattern in 2 of 8 cases (in 2 of them there was only a slight difference between the kidneys) and with respect to the urinary concentration of Na in 2 of 11 cases (in 5 determinations the difference was ≤ 15 per cent). Table V. Schlegel *et al* (220) however maintained that the urinary flow in the ischaemic kidney was lower but that the Na concentration in urine was the same in both kidneys in patients with hypertension which improved after nephrectomy. Birchall *et al* (22) reported two cases in which the urinary flow was greatly reduced on the affected side. In one of these patients the Na concentration in urine was higher for the ischaemic than for the non ischaemic kidney. This particular finding was confirmed by Hulet *et al* (109).

Kjellbo *et al* (134) also reported that the Na concentration in urine from the stenosed kidney was moderately depressed compared to that from the other kidney. The present series contains too few cases to permit any conclusions based on the urinary concentration of Na or creatinine.

A lower RPF on the stenosed side has also been reported (65, 240). This was found to apply to two of the 4 patients in the present study in whom this function could be determined.

The *urina* and creatinine clearances were found by Brown *et al* (34) to be higher on the stenosed side whereas Kjellbo *et al*

been demonstration of the extent of the pyelonephritic process in the tubules of each kidney — as far as this can be localized by means of the various tests available. This is exemplified by the following case.

CASE 27 a 21 year old woman. She fulfilled 4 of the 8 diagnostic criteria tested. She had a history of cystitis with and without fever as well as indefinite febrile attacks without signs of cystitis and a feeling of tension in the left flank. On admission to hospital palpation over this flank elicited tenderness. The left kidney was found to have poorer concentration ability than the right, and at renal vein catheterization the PAH extraction was lower on the left side. These findings were compatible with the tension in the left flank and the tenderness to palpation. Although compilation of the findings strongly supported the suspicions of chronic pyelonephritis the possibility of recurrent acute pyelonephritis could not be entirely ruled out.

Other examples have already been given of cases in which split function tests contributed to the diagnosis of chronic pyelonephritis (cf pp 109—110).

Renal disease (except pyelonephritis) with unilateral or mainly asymmetric involvement (group B2)

In the present series most cases in this group of renal diseases showed a corresponding asymmetry in the various split function tests to that in groups A1, A2 and B1.

Separate renal function tests combined with for instance roentgenologic examination have proved to be particularly valuable in patients in whom involvement by the relevant disease seems to be unilateral and operation is being considered. The following case in the present series can serve as an example.

CASE 107 an 18 year old woman. Diagnosis dysplasia of left kidney + arterial hypertension.

Four years before the current investigation she had scarlet fever with proteinuria. Urine analyses 2 years later showed nothing abnormal. Examination in 1955 disclosed arterial hypertension (195/140 mm Hg) and she was therefore hospitalized. She had no subjective complaints apart from periodic headaches.

The 24-hour blood pressure ranged from 207/120 to 150/100 mm Hg (mean 179/113 mm). Ocular fundi changes corresponding to FH I. Roentgenologic examination of the heart size normal. ECG: signs of myocardial anoxaemia. Excretory pyelography showed the left kidney to be considerably smaller than the right, the density of contrast medium in it was impaired and excretion delayed. Renal aortography disclosed that the left main renal artery was no wider than a match although its walls showed no signs of local stenosis.

In view of these findings nephrectomy was considered. Bilateral renal vein and ureteric catheterization were carried out to assess the function of each kidney and hence the patient's chances of survival with only one. The results clearly showed that the right kidney had largely normal function apart from a slight reduction in concentration ability (534 mOsmol/lit). The difference between the kidneys was marked with respect to clearances and PAH extraction and less pronounced with respect to concentration and acidifying ability. Left sided nephrectomy was performed and histologic examination of the kidney showed malformation of the type described by Ask-Upmark (3) together with slight chronic pyelitis. The patient has had normal blood pressure at several subsequent controls of which the most recent was a 24 hour recording in June 1965.

Arterial hypertension with stenosis or an aneurysm of the main renal artery (group B3)

Arterial hypertension with involvement of the main renal artery is represented in the present study by 13 cases of unilateral stenosis of this artery, one of bilateral stenosis and one case of bilateral aneurysm of the artery.

term therapy in the intervals between them, and regular check ups

As stated earlier, the total renal function can in early stages of *e.g.* pyelonephritis show normal values this is because the function of the better kidney masks the functional defect of the affected one. This circumstance can be detected only by means

of bilateral studies of renal function (*cf* p 59)

In cases of renal disease in which routine examinations do not provide a definite diagnosis or in which the indications for operation are not clear demonstration of the function of each kidney separately is often of great value

(134) and Dustan *et al* (65) stated that the GFR was markedly reduced on the stenosed side. In the present study, the GFR — determined from the endogenous creatinine clearance — was lower on the stenosed side in 6 of the 9 cases in which it was compared and higher in 2.

The bilateral study of renal function helped to clarify the indications for operation in most of my cases of hypertension and stenosis of the main renal artery. The 7 cases in which the diagnosis could be verified at operation are noted in Table IV.

As already pointed out, some stenosed kidneys excrete more concentrated urine and in most cases there is a retention of Na, unlike the picture in chronic pyelonephritis. Cases are, of course, found with a combination of both diseases, one of which will then predominate in the functional pattern (cf p 101).

No typical functional pattern was displayed by the few patients in the present study who had some other renal disease than chronic obstructive and non obstructive pyelonephritis or hypertension with stenosis of the main renal artery.

Renal disease with presumed symmetric involvement of both kidneys (group B4) and cases with lower urinary tract symptoms in which renal involvement could not be ruled out (group B5)

In these patients — who were presumed to have relatively homogeneous involvement of the kidneys (group B4) or intact kidneys (group B5) — the various functions corresponded on the whole to the expectations. The symmetry of renal function in cases of essential hypertension has been described by, e.g. Dustan *et al* (65) and Hulet *et al* (109).

Conclusions

On the basis of the present study it can be stated that asymmetric renal function can be demonstrated in many cases of chronic pyelonephritis, as well as of such diseases which obviously affect one kidney more than the other. In these cases tests for asymmetry can support the diagnosis or facilitate the choice of therapy although they are seldom necessary for adequate evaluation of the case. On the other hand, a lack of asymmetry does not argue against pyelonephritis when other symptoms and signs strongly suggest the presence of this disease. In diagnostically doubtful cases however, the absence of asymmetry may indicate the existence of another type of disease which involves the kidneys more homogeneously. Naturally when taking into account the existence of asymmetry it does not suffice to establish whether one or another of the renal functions is asymmetric. The degree of asymmetry must also be considered. This is of decisive importance in certain cases e.g. if one of the kidneys has ceased to function.

In cases of predominantly unilateral acute pyelonephritis a marked reduction in various functions is often found in the affected kidney if separate functional tests are made in connexion with the acute onset. Normal values are frequently recorded for the other kidney. If these tests are repeated some time later the functional impairment of the affected kidney will either have been restored to the normal level or will persist. In the latter event one often finds not only successive deterioration of function in this kidney but also gradual signs of similar damage to the contralateral kidney. This development can however — as previously illustrated (cf p 112) — be arrested by intensified treatment of acute attacks combined with long

term therapy in the intervals between them, and regular check ups

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SUMMARY

Chapter I Criteria of Asymmetry

The physiologic symmetry in function of the two kidneys is discussed. On the basis of the percentage difference between the functional capacity of the kidneys with respect to the individual parameters borderlines are drawn for the values regarded as indicative of asymmetric function.

Chapter II Earlier Bilateral Comparisons Between Healthy Kidneys

A survey is given of investigations by other authors in which the function of the two kidneys was compared in healthy subjects.

Chapter III Diagnosis of Chronic Pyelonephritis

The diagnosis of chronic pyelonephritis is discussed. Eight cardinal diagnostic criteria are set up. In the present series a diagnosis of chronic pyelonephritis is made only when at least three of these criteria are fulfilled.

Chapter IV Diagnosis of Other Renal Diseases

The diagnosis of the renal diseases other than pyelonephritis — which are included in the present series for comparative purposes — is discussed.

Chapter V Case Material

The present investigation is based on 152 patients with renal diseases consisting in 80

cases of chronic or acute non obstructive pyelonephritis (groups A1 A2). The remaining 72 patients consisted of 25 with chronic pyelonephritis and urinary tract obstruction (group B1), 11 with unilateral or mainly asymmetric renal damage (group B2), 15 with arterial hypertension and stenosis or aneurysm of the main renal artery (group B3), 16 with renal damage which could be expected to be bilaterally homogeneous (group B4), and 5 in whom renal involvement could not be ruled out (group B5). The comparative material comprised 11 volunteers with healthy kidneys in whom the renal extraction of para aminohippuric acid (PAH) was determined.

Chapter VI Choice of Methods for Demonstrating Asymmetry

A brief survey is given of the methods available for demonstrating asymmetry of renal function. In the present series bilateral renal vein catheterization and/or bilateral aortic catheterization were performed in every case. This permitted the renal extraction of para aminohippuric acid (PAH) and the renal concentration ability to be determined in each kidney separately. In several cases other split function tests were also made.

Chapter VII Methods

The methods used in the present investigation are described. With respect to renal vein catheterization the advantage is stressed

of using closed-circuit television for guiding the catheter — In a few cases the renal extraction of radioactive Diodrast by each kidney was studied in addition to that of PAH

Chapter VIII Sources of Error in the Clinical Physiologic Methods

The sources of error in the methods used are discussed from both the general and the physiologic aspects. Particular attention is focused on the extent to which renal function may be influenced by the introduction of ureteric catheters as well as on the risk of leakage of urine outside the catheters.

Chapter IX Results

The results of split function tests are given chiefly in tabular form. In the subjects with healthy kidneys the extraction ability was invariably symmetric. In most cases of pyelonephritis with impaired renal function the reduction was asymmetric in several parameters tested. As a rule the asymmetry was greatest in patients with moderately impaired function. When the kidneys were severely damaged the reduced functional capacity tended to become symmetric again. In general the asymmetry of the renal concentration ability was greater than that of the extraction ability.

Furthermore in chronic pyelonephritis the concentration ability was usually more reduced than the extraction ability. The glomerular filtration rate was generally less reduced than the concentration ability although it was more affected than the extraction ability.

In the cases of uncomplicated pyelonephritis the renal extraction ability — but not the other parameters — was on the

whole significantly lower on the left side than on the right.

In five cases of chronic pyelonephritis the extraction ability of each kidney was studied simultaneously at varying plasma concentrations of PAH. The difference between the depression limit in the two kidneys was recorded in two of the cases.

The results were less consistent in the patients with renal diseases other than chronic and acute pyelonephritis. However in most cases of stenosis of the main renal artery the existence of asymmetry with respect to urinary volume, osmolality and sodium concentration was in agreement with the reports of other authors.

Chapter X General Discussion

A Implications of asymmetry of individual functions. The fact that in cases of pyelonephritis the PAH extraction by the left kidney was as a rule more reduced than that by the right is discussed. Various explanations of this observation are suggested.

B Relation between the types of functional damage and their pathophysiologic implications in the individual kidney. In moderately severe pyelonephritis the renal concentration ability was generally more reduced than the extraction. This was interpreted as an indication that the medulla was more damaged than the cortex. In cases of acute pyelonephritis in which renal damage was slight the degree of functional impairment was about the same for both parameters. The fact that this also applied in extremely severe pyelonephritis is ascribed to both the medulla and the cortex being so seriously damaged that only an appreciable functional capacity remained in both.

organs. Various alternatives are discussed to explain why the medulla is more sensitive to pyelonephritic damage.

C Interpretation of a decreased extraction ability Bilateral determinations were made of the upper limit of the PAH concentration in blood at which the PAH extraction no longer remained constant, but started to fall — the depression limit. This permitted conclusions about whether damage to the proximal tubules was due to a regional loss of their PAH transporting cells, or to a general decrease in the capacity of these cells to transport PAH.

D Clinical value of testing for asymmetry of renal function Only in relatively few

cases did demonstration of asymmetry with respect to concentration and extraction ability play a decisive role in diagnosis of a renal disease. However — as pointed out by other authors — demonstration of asymmetry was, in fact, often a prerequisite for evaluating the indications for operation in urological cases. This could be confirmed particularly as regards urinary volume and urinary sodium concentration in stenosis of the main renal artery.

During the course of a renal disease, the individual renal functions could be followed in each kidney separately. This was found to be of special interest in long term treatment of chronic pyelonephritis which as a rule led to improvement.

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